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(54) **The gal operon of streptomyces.**

(57) A recombinant DNA molecule comprising the *Streptomyces gal* operon *galK* gene; *galE* gene; *galT* gene; P1 promoter; P2 promoter; P2 promoter expression unit; P1 promoter regulated region; or the entire *Streptomyces gal* operon is prepared.

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TITLE
THE GAL OPERON OF
STREPTOMYCES

CROSS REFERENCE TO RELATED APPLICATIONS

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This application is a continuation-in-part of Serial Number 834,706, filed February 28, 1986, which is pending.

BACKGROUND OF THE INVENTION

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This invention relates to a recombinant DNA molecule comprising the Streptomyces gal operon.

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Hodgson, J. Gen. Micro., 128, 2417-2430 (1982), report that Streptomyces coelicolor A3(2) has a glucose repression system which allows repression at the level of transcription of the arabinose uptake system, one of the glycerol uptake systems, and also repression of the galactose uptake system in wild type strains. There is no report in Hodgson of actual galactose metabolism by S. coelicolor A3(2).

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Okeda et al. Mol. Gen. Genet., 196, 501-507 (1984), report that glucose kinase activity, 2-deoxyglucose-sensitivity, glucose utilization and glucose repression were all restored to S. coelicolor A3(2) glk (glucose kinase) mutants transformed by a 3.5 kb DNA fragment which contained the glk gene cloned from S. coelicolor into a phage vector.

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1 Seno et al., Mol. Gen. Genet., 193, 119-128
(1984), report the glycerol (gyl) operon of Streptomyces
coelicolor, and state that such operon is substrate-
inducible and catabolite-repressible.

5 Debouck et al., Nuc. Acids. Res., 13(6), 1841-1853
(1985), report that the gal operon of E. coli consists of
three structurally contiguous genes which specify the
enzymes required for the metabolism of galactose, i.e.,
10 galE (uridine diphosphogalactose-4-epimerase), galT
(galactose-1-phosphate uridylyltransferase) and galK
(galactokinase); that such genes are expressed from a
polycistronic mRNA in the order E, T, K; that the
expression of the promoter distal gene of the operon,
15 galK, is known to be coupled translationally to the galT
gene immediately preceding it; that such translational
coupling results from a structural overlap between the end
of the galT coding sequence and the ribosome binding
region of galK; and that the translational coupling of
20 galT and galK ensures the coordinate expression of these
genes during the metabolism of galactose.

SUMMARY OF THE INVENTION

This invention relates to a recombinant DNA
molecule comprising a Streptomyces gal operon galK gene;
galE gene; galT gene; P2 promoter expression unit, or P2
25 promoter or any functional derivative thereof as well as a
recombinant DNA molecule comprising a Streptomyces gal
operon P1 promoter, P1 promoter regulated region or the
entire gal operon or any regulatable and functional
derivative thereof.

30 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon or any
regulatable and functional derivative thereof and a
functional DNA molecule operatively linked to such operon;
a recombinant DNA vector comprising and such DNA molecule,
35 and, optionally, additionally comprising a replicon; a
method of preparing a host cell transformed with such

1 vector; the transformed host prepared by such method; a
method of expressing such functional DNA sequence which
comprises cultivating such transformed host under suitable
5 conditions such that the functional DNA sequence is
expressed; and to a method of regulating the expression of
such functional DNA sequence which comprises cultivating
such transformed host under conditions which regulate such
expression.

10 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter expression unit or any functional derivative
thereof and a functional DNA molecule operatively linked
to such unit; a recombinant DNA vector comprising such DNA
15 molecule, and, optionally, additionally comprising a
replicon; a method of preparing a host cell transformed
with such vector; the transformed host prepared by such
method; and to a method of expressing such functional DNA
sequence which comprises cultivating such transformed host
20 under suitable conditions such that the functional DNA
sequence is expressed.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P1
promoter regulated region or any regulatable and
functional derivative thereof and a functional DNA
25 molecule operatively linked to such region; a recombinant
DNA vector comprising such DNA molecule, and, optionally,
additionally comprising a replicon; a method of preparing
a host cell transformed with such vector; the transformed
host prepared by such method; a method of expressing such
30 functional DNA sequence which comprises cultivating such
transformed host under suitable conditions such that the
functional DNA sequence is expressed; and to a method of
regulating the expression of such functional DNA sequence
which comprises cultivating such transformed host under
35 conditions which regulate such expression.

This invention also relates to a recombinant DNA

1 molecule comprising the Streptomyces gal operon P1
promoter or any regulatable and functional derivative
thereof and a foreign functional DNA molecule operatively
linked to such region; a recombinant DNA vector comprising
5 such DNA molecule, and, optionally, additionally
comprising a replicon; a method of preparing a host cell
transformed with such vector; the transformed host
prepared by such method; a method of expressing such
functional DNA sequence which comprises cultivating such
10 transformed host under suitable conditions such that the
functional DNA sequence is expressed; and to a method of
regulating the expression of such functional DNA sequence
which comprises cultivating such transformed host under
conditions which regulate such expression.

15 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter or any functional derivative thereof and a
foreign functional DNA molecule operatively linked to such
region; a recombinant DNA vector comprising such DNA
20 molecule, and, optionally, additionally comprising a
replicon; a method of preparing a host cell transformed
with such vector; the transformed host prepared by such
method; and to a method of expressing such functional DNA
sequence which comprises cultivating such transformed host
25 under suitable conditions such that the functional DNA
sequence is expressed.

This invention also relates to a method of
enabling a non-galactose utilizing host microorganism or
cell to utilize galactose which comprises transforming
30 such host with a recombinant DNA molecule comprising a
Streptomyces gal operon or any portion of the Streptomyces
gal operon, or any functional derivative thereof, which is
adequate to enable such transformed host to utilize
galactose, This invention also relates to the recombinant
35 DNA vector employed in such method and to the host
prepared by such method.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a restriction endonuclease map of the Streptomyces lividans 1326 galactose (gal) operon and indicates approximate locations for structural genes and promoters within the operon.

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Figure 2 represents a restriction endonuclease map of plasmid pK21.

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Figure 3 represents a comparison of the restriction endonuclease maps of the S. lividans gal operon and a restriction fragment containing the S. coelicolor galK gene.

DETAILED DESCRIPTION OF THE INVENTION

15

It has now been discovered that the Streptomyces genome contains a operon for the metabolism of galactose (i.e., a gal operon) which comprises three structural genes (galT, galE and galK) and two promoters (P1 and P2). The galT gene product is known as galactose-1-phosphate uridyltransferase (transferase), the galE gene product is known as uridine diphosphogalactose-4-epimerase (epimerase), and the galK gene product is known as galactose-1-kinase (galactokinase). The function of the gene products of galT, galE and galK in galactose metabolism in Streptomyces is explained by the following diagram:

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1. galactose + ATP galactokinase
galactose-1-phosphate + ADP
2. galactose-1-phosphate + UDP-glucose transferase
30 UDP-galactose + glucose-1-phosphate
3. UDP-galactose epimerase UDP-glucose

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By the term "promoter" is meant any region upstream of a structural gene which permits binding of RNA polymerase and transcription to occur.

1 By the term "structural gene" is meant a coding sequence for a polypeptide which serves to be the template for the synthesis of mRNA.

5 By the term "operon" is meant a group of closely linked genes responsible for the synthesis of one or a group of enzymes which are functionally related as members of one enzyme system. An operon comprises an operator gene, a number of structural genes (equivalent to the number of enzymes in the system) and a regulator gene. By

10 "operator" or "operator gene" is meant a DNA sequence which controls the biosynthesis of the contiguous structural gene(s) within an operon. By "regulator gene" is meant a gene which controls the operator gene in an

15 operon through the production of a repressor which can be either active (enzyme induction) or inactive (enzyme repression). The transcription of the structural gene(s) in an operon is switched on or off by the operator gene

20 which is itself controlled in one or more of three ways: 1) in inducible enzyme systems, the operator is switched off by a repressor produced by the regulator gene and

25 which can be inactivated by some metabolite or signal substance (an inducer) coming from elsewhere in the cell or outside the cell, so that the presence of the inducer results in the operon becoming active; or 2) in repressed

30 enzyme systems, the operator is switched off by a repressor-corepressor complex which is a combination of an inactive repressor produced by the regulator gene with a corepressor from elsewhere, so that the presence of the

35 corepressor renders the operon inactive; or 3) in activated gene systems, the promoter is switched on by an activator produced by a regulator gene which can be

activated by some metabolic or signal substance.

The Streptomyces gal operon is naturally present in the Streptomyces genome.

By the term "Streptomyces gal operon" is meant that region of the Streptomyces genome which comprises the

1 P1 promoter, P2 promoter, galT, galE and galK structural
genes and any other regulatory regions required for
transcription and translation of such structural genes.

5 By the term "regulatory region" is meant a DNA
sequence, such as a promoter or operator, which regulates
transcription of a structural gene.

The following model is suggested for gene
expression within the Streptomyces gal operon. The P1
10 promoter is a galactose inducible promoter (i.e., it is
induced in the presence of galactose and repressed in the
presence of glucose). According to S1 data, the P2
promoter is constitutive, i.e., it is "turned on"
regardless of the presence or absence of galactose or any
other carbon source.

15 A cosmid library was constructed for Streptomyces
lividans 1326 DNA by using cosmid pJW357 (which encodes
the ability to replicate in both Streptomyces and E.
coli). This library was then transfected into E. coli K21
20 which is a derivative of the E. coli strain MM294 which
contained a bacteriophage P1 transduced galactokinase
(galK) mutation. Transfected cells were plated under
media conditions which select for both the presence of the
cosmid and the presence of an active galK gene. Weakly
positive colonies were isolated and the cosmid DNA derived
25 from these colonies was transformed into the K21 strain.
These transformations yielded two cosmids which
consistently produced positive growth with galactose as
the only carbon source. These galK⁺ cosmids were then
transformed into a Streptomyces host (i.e., Streptomyces
30 lividans 1326-12K) which had been isolated by the
inventors of the subject invention as unable to grow on
medium in which galactose was the only carbon source by
using 2-deoxy-galactose selection [see, Brawner et al.,
Gene, 40 191 (1985), in press]. Under conditions which
35 differentiate strains able and unable to produce

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galactokinase, only one of the cosmids caused the Streptomyces lividans 1326-12K host to become galK⁺.

5 Further studies have demonstrated that this cosmid encodes a gene with galactokinase activity. Additional studies, including DNA sequence analysis and protein studies demonstrate that this Streptomyces gene shares homology with the E. coli and yeast galactokinase genes. Regulation studies indicate that the cosmid encoded

10 galactokinase gene regulated in the same manner as the chromosome encoded gene.

A. S. lividans gal operon was originally isolated from a ca. 9 kilobase (Kb) region of Streptomyces lividans 1326. The ca. 9 Kb region of Streptomyces lividans 1326

15 containing the Streptomyces gal operon has been mapped substantially as follows in Table A. By "substantially" is meant (i) that the relative positions of the restriction sites are approximate, (ii) that one or more restriction sites can be lost or gained by mutations not

20 otherwise significantly affecting the operon, and (iii) that additional sites for the indicated enzymes and, especially for enzymes not tested, may exist. The restriction enzymes used herein are commercially available. All are described by Roberts, Nuc. Acids.

25 Res., 10(5): p117 (1982).

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TABLE A

	<u>Map Position</u>	<u>Restriction Enzyme</u>	<u>Location (kb)</u>
5	1	<u>HindIII</u>	-.40
	1a	<u>NruI</u>	0
	2	<u>BglII</u>	.75
	3	<u>EcoRI</u>	1.05
	4	<u>PvuII</u>	1.15
10	5	<u>MluI</u>	2.30
	6	<u>PvuII</u>	2.80
	7	<u>EcoRI</u>	4.00
	8	<u>PvuII</u>	4.10
	8a	<u>SacI</u>	4.25
15	9	<u>PvuII</u>	5.00
	10	<u>XhoI</u>	5.50
	11	<u>BamHI</u>	5.80
	12	<u>BamHI</u>	6.50
	13	<u>MluI</u>	6.90
20	13a	<u>PvuII</u>	7.20
	14	<u>MluI</u>	7.80
	15	<u>BamHI</u>	8.00
	16	<u>SphI</u>	8.30

25 Figure 1 represents a restriction endonuclease map of the Streptomyces lividans 1326 gal operon and indicates locations for structural genes (galT, galE and galK) and promoters (P1 and P2) comprised within the operon.

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35

Referring to Table A and Figure 1, the location of the promoters and structural genes of the Streptomyces lividans 1326 gal operon are mapped substantially as follows in Table B:

TABLE B

	<u>Location (Kb)</u>
P1 transcription start site	.10
<u>galT</u> translation initiation codon	.15
P2 transcription start site	1.25
<u>galE</u> translation initiation codon	1.50
<u>galK</u> translation initiation codon	2.40
3' end of <u>galK</u> message	3.60

Microorganisms of the genus Streptomyces have historically been used as a source of antibiotics for the pharmaceutical industry. Consequently, the technical skills necessary to scale-up the production of biological products using Streptomyces as the vehicle for the production of such products are presently available. However, before Streptomyces can be used as a vehicle for the production of bioactive molecules using the new recombinant DNA technologies, there is a need to define regulatory elements in Streptomyces analogous to those which have proved useful in E. coli. These regulatory elements include ribosomal binding sites and regulated transcriptional elements.

The existence of a galE, galT or galK gene or gene product or gal operon in Streptomyces has not been previously reported. The instant invention, i.e., the cloning of the Streptomyces gal operon, enables construction of regulatable expression/cloning vectors in Streptomyces, other actinomycetes, and other host organisms. Furthermore, the instant invention led to the discovery that the Streptomyces gal operon is

1 polycistronic. Perhaps the most important feature of the
cloning of the Streptomyces gal operon is the observation
that there are sequences essential for regulation of the
Streptomyces galK gene. Direct analogy to the initial use
5 of the lac promoter from E. coli as an expression system
can be made. In fact, Brosius et al., Proc. Natl. Acad.
Sci. USA, 81, 6929-6933 (1984), utilized the regulatory
elements of the E. coli lac promoter to regulate the
exceptionally strong E. coli ribosomal promoters. Because
10 it is likely that the Streptomyces gal operon ribosomal
promoters are also exceptionally strong, such promoters
enable the construction of regulatable expression vectors
which will be very useful in Streptomyces, other
actinomycetes, and other host organisms. The instant
15 invention also enabled the unexpected discovery that the
2-deoxygalactose selection which has been used in E. coli
to select for galK mutants also operates in Streptomyces
to select for galK mutants [see, Brawner et al., Gene 40,
191 (1985), in press]. This observation, combined with
20 the ability to clone the Streptomyces galK gene and the
promoter and regulatory regions required for its
transcription and translation on a cosmid, as described
herein, allows the direct insertion of any structural gene
into the chromosomally located galK gene of Streptomyces
25 by homologous recombination. This manipulation will allow
molecular biologists to stably insert DNA fragments of
interest into the Streptomyces chromosome. Such an
approach will allow researchers to tag or mark a
Streptomyces strain of interest or to insert expression
30 cassettes into the organism without the need of
maintaining an antibiotic selection such as that presently
required by most Streptomyces expression vectors.

This invention relates to a recombinant DNA
molecule comprising the Streptomyces gal operon or any
35 regulatable and functional derivative thereof.

1 By "regulatable and functional derivative" is meant any
derivative of the Streptomyces gal operon which functions
in substantially the same way as the naturally occurring
Streptomyces gal operon in terms of regulatable production
5 of the galT, galE and galK gene products. Such
derivatives include partial sequences of the gal operon,
as well as derivatives produced by modification of the gal
operon coding sequence. Techniques for modifying the gal
operon which are known in the art include, for example,
10 treatment with chemical mutagens, irradiation or direct
genetic engineering, such as by inserting, deleting or
substituting nucleic acids by the use of enzymes or
recombination techniques. The naturally occurring
Streptomyces gal operon can be isolated from any galactose
15 utilizing Streptomyces strain by employing the techniques
described herein. Numerous strains of various
Streptomyces species are publicly available from many
sources. For example, the American Type Culture
Collection, Rockville, Maryland, U.S.A. has approximately
20 400 different species of Streptomyces available to the
public. The ability of a particular strain of
Streptomyces to utilize galactose can be readily
determined by conventional techniques, such as by growing
such strain on a medium containing galactose as the sole
25 carbon source. The preferred Streptomyces species from
which to isolate a gal operon include S. lividans, S.
coelicolor, S. azuraeus and S. albus, S. carzinostaticus,
S. antifibrinolyticus and S. longisporus. S. lividans is
most preferred. The Streptomyces gal operon, and smaller
30 portions thereof, is useful as a nucleic acid probe to
obtain homologous sequences from other cells and
organisms. The Streptomyces gal operon is also useful as
a selection marker in an appropriate host mutant, and for
providing regulatory elements. By "appropriate host
35 mutant" is meant a host which does not utilize galactose

1 because it (a) does not contain a gal operon or (b)
contains a nonfunctional gal operon, or (c) contains a
defect within a homologous structural gene or regulatory
region comprised by the Streptomyces gal operon such as a
5 defective P1 promoter, P2 promoter, galT gene, galK gene
and/or galE gene. Thus, a recombinant DNA molecule
(comprising the Streptomyces gal operon and a foreign
functional DNA sequence operatively linked thereto), which
can be prepared by conventional techniques, can be
10 transformed into an appropriate host mutant by
conventional techniques for incorporation into the host
genome by homologous recombination to enable regulatable
expression of the foreign functional DNA sequence without
the need of maintaining an expensive antibiotic
15 selection. Such operon may therefore also be incorporated
on recombinant DNA expression vectors for regulatable
expression of a foreign functional DNA sequence
operatively linked to such operon in an appropriate host
mutant transformed with such vector without the need of
20 maintaining an expensive antibiotic selection. Such
operon is also useful for transforming those cells,
viruses and microorganisms, such as strains of
Streptomyces, other actinomycetes, and other prokaryotic
organisms, such as gal⁻ E. coli strains, which do not
25 utilize galactose into galactose utilizing strains. Such
transformation may have pleiotrophic effects on the
transformed host. By the term "functional DNA sequence"
is meant any discrete region of DNA derived directly or
indirectly from Streptomyces or any other source which
30 functions in a host organism transformed therewith as a
gene expression unit, structural gene, promoter or a
regulatory region. Preferred functional DNA sequences
include those coding for polypeptides of pharmaceutical
importance, such as, but not limited to, insulin, growth
35 hormone, tissue plasminogen activator, alpha -1-anti-
trypsin or antigens used in vaccine production. By the

1 term "foreign functional DNA sequence" is meant a
functional DNA sequence not derived from the Streptomyces
gal operon coding region.

5 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter expression unit or any functional derivative
thereof. By the term "P2 promoter expression unit" is
meant that region of the Streptomyces gal operon
10 comprising the Streptomyces gal operon P2 promoter, galE
and galK structural genes and any other regulatory regions
required for transcription and translation of such
structural genes. By "functional derivative" is meant any
derivative of the Streptomyces gal operon P2 promoter
15 expression unit which functions in substantially the same
way as the naturally occurring region in terms of
production of the Streptomyces gal operon galE and galK
gene products. Such derivatives include partial sequences
of the Streptomyces gal operon P2 promoter expression
20 unit, as well as derivatives produced by modification of
the Streptomyces gal operon P2 promoter expression unit
coding sequence. Techniques for effecting such
modification are known in the art, and some have been
outlined above. The naturally occurring Streptomyces gal
operon P2 promoter expression unit can be isolated from
25 the naturally occurring Streptomyces gal operon by
conventional techniques. The Streptomyces gal operon P2
expression unit is useful as a selection marker in an
appropriate host mutant and for providing regulatory
elements. By "appropriate host mutant" is meant a host
30 which does not utilize galactose because it contains a
defect within a homologous structural gene or regulatory
region comprised by the Streptomyces P2 promoter
expression unit such as a defective P2 promoter, galE gene
and/or galK gene. Thus, a recombinant DNA molecule
35 (comprising the Streptomyces gal operon P2 promoter
expression unit and a foreign functional DNA sequence .

1 operatively linked thereto), which can be prepared by
conventional techniques, can be transformed into an
appropriate host mutant by conventional techniques for
incorporation into the host genome by homologous
5 recombination to enable constitutive expression of the
foreign functional DNA sequence without the need of
maintaining an expensive antibiotic selection. Such
expression unit may also be incorporated on recombinant
DNA expression vectors for constitutive expression of
10 foreign functional DNA sequences. The Streptomyces gal
operon P2 promoter expression unit is also useful for
complementation of an appropriate host mutant which can
then be used for constitutive expression of a foreign
functional DNA sequence operatively linked to such
15 expression unit in an appropriate host mutant transformed
with such vector without the need of maintaining an
expensive antibiotic selection.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P1
20 promoter regulated region or any regulatable and
functional derivative thereof. By the term "P1 promoter
regulated region" is meant that region of the Streptomyces
gal operon comprising the Streptomyces gal operon P1
promoter, galT, galE and galK structural genes and any
25 other regulatory regions required for transcription and
translation of such structural genes. By "regulatable and
functional derivative" is meant any derivative of the
Streptomyces gal operon P1 promoter regulated region which
functions in substantially the same way as the naturally
30 occurring region in terms of regulatable production of the
Streptomyces gal operon galT, galE and galK gene
products. Such derivatives include partial sequences of
the Streptomyces gal operon P1 promoter regulated region,
as well as derivatives produced by modification of the
35 Streptomyces gal operon P1 promoter regulated region
coding sequence. Techniques for effecting such

1 modification are known in the art, and some have been
outlined above. The naturally occurring Streptomyces gal
operon P1 promoter regulated region can be isolated from
the naturally occurring Streptomyces gal operon by
5 conventional techniques, such as by excising the P2
promoter from the naturally occurring Streptomyces gal
operon or inactivating the P2 promoter by a point mutation
or by inserting a foreign DNA sequence within the
promoter. The Streptomyces gal operon P1 promoter
10 regulated region is useful for the utilities outlined
above for the Streptomyces gal operon.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter or any functional derivative thereof. By
"functional derivative" is meant any derivative of the
15 Streptomyces gal operon P2 promoter which functions in
substantially the same way as the naturally occurring P2
promoter in terms of enabling the binding of RNA
polymerase thereto and transcription of a functional DNA
sequence operatively linked to such promoter. Such
20 derivatives include partial sequences of the Streptomyces
gal operon P2 promoter, as well as derivatives produced by
modification of the gal operon P2 promoter coding
sequence. Techniques for effecting such modification are
known in the art, and some have been outlined above. The
25 naturally occurring Streptomyces gal operon P2 promoter
can be isolated from the naturally occurring Streptomyces
gal operon by conventional techniques. A recombinant DNA
molecule (comprising the Streptomyces gal operon P2
promoter and a foreign functional DNA sequence operatively
30 linked thereto), which can be prepared by conventional
techniques, can be transformed into an appropriate host
mutant by conventional techniques for incorporation into
the host genome by homologous recombination to enable
constitutive expression of the foreign functional DNA
35 sequence. The Streptomyces gal operon P2 promoter is also

1 useful for incorporation into recombinant DNA expression
vectors for constitutive expression of a foreign
functional DNA sequence operatively linked thereto in
viruses and eukaryotic or prokaryotic cells or organisms,
5 especially in Streptomyces or other actinomycetes,
transformed with such vector.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P1
promoter or any regulatable and functional derivative
10 thereof. By "regulatable and functional derivative" is
meant any derivative of the Streptomyces gal operon P1
promoter which functions in substantially the same way as
the naturally occurring P1 promoter in terms of enabling
the binding of RNA polymerase thereto and regulating the
15 transcription of a functional DNA sequence operatively
linked to such promoter. Such derivatives include partial
sequences of the Streptomyces gal operon P1 promoter, as
well as derivatives produced by modification of the gal
operon P1 promoter coding sequence. Techniques for
20 effecting such modification are known in the art, and some
have been outlined above. The naturally occurring
Streptomyces gal operon P1 promoter can be isolated from
the naturally occurring Streptomyces gal operon by
conventional techniques. A recombinant DNA molecule
25 (comprising the Streptomyces gal operon P1 promoter and a
foreign functional DNA sequence operatively linked
thereto), which can be prepared by conventional
techniques, can be transformed into an appropriate host
mutant by conventional techniques for incorporation into
30 the host genome by homologous recombination to enable
regulatable expression of the foreign functional DNA
sequence. The Streptomyces gal operon P1 promoter is also
useful for incorporation into recombinant DNA expression
vectors for regulatable expression of a foreign functional
35 DNA sequence operatively linked thereto in viruses and
eukaryotic or prokaryotic cells or organisms, especially

1 Streptomyces or other actinomycetes, transformed with such vector.

5 This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon galE, galT or galK gene, or any functional derivative thereof. By "functional derivative" is meant any derivative of the Streptomyces gal operon galE, galT or galK gene which functions in substantially the same way as the naturally occurring gene in terms of production of an active galE, galT, or galK type gene product. Such derivatives include partial sequences of the Streptomyces gal operon galE, galT, or galK gene, as well as derivatives produced by modification of the gal operon sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon galE, galT and/or galK gene can be isolated from the naturally occurring Streptomyces gal operon by conventional techniques. The Streptomyces gal operon galE, galT and/or galK gene can be used as a selection marker in an appropriate host mutant. By "appropriate host mutant" is meant a host which does not utilize galactose because it contains a defect within a homologous galE, galT and/or galK gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon galE, galT and/or galK gene and a foreign functional DNA sequence, both of which are operatively linked to appropriate regulatory region), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable detection of transformants without the need of maintaining an expensive antibiotic selection. Likewise, a recombinant DNA vector comprising the Streptomyces gal operon galE, galT and/or galK gene and a foreign functional DNA sequence, both of which are operatively linked to appropriate regulatory

1 regions, as well as a replicon, can be transformed into an
appropriate host mutant by conventional techniques to
enable detection of transformants without the need of
maintaining an expensive antibiotic selection. The
5 Streptomyces gal operon galE, galK and/or galT gene is
also useful for complementation of an appropriate host
mutant.

The Streptomyces gal operon galE gene is also
useful for providing a ribosome binding site and
10 initiation codon which can be fused to a foreign
functional DNA sequence to enable the expression of such
coding sequence when incorporated into an appropriate
expression vector and transformed into an appropriate
host. If such foreign functional DNA sequence is fused to
15 the galE gene ribosome binding site and initiation codon
in a recombinant DNA expression vector comprising the
Streptomyces gal operon P2 promoter expression unit, or
the entire gal operon, such DNA sequence will be
constitutively expressed when such vector is transformed
20 into an appropriate host organism. If such DNA sequence
is fused to the galE gene ribosome binding site and
initiation codon in a recombinant DNA expression vector
comprising the Streptomyces gal operon P2 promoter
regulated region, expression of such DNA sequence can be
25 regulated when such vector is transformed into an
appropriate host organism by controlling the presence or
absence of galactose or glucose.

The Streptomyces gal operon galT gene is also
useful for providing a ribosome binding site and
30 initiation codon which can be fused to a foreign
functional DNA sequence to enable the expression of such
coding sequence when incorporated onto an appropriate
expression vector and transformed into an appropriate
host. If such DNA sequence is fused to the galT gene
35 ribosome binding site and initiation codon in a
recombinant DNA expression vector comprising the

1 Streptomyces gal operon P1 promoter regulated region, or
the entire gal operon, expression of such coding sequence
can be regulated in a host transformed with such vector as
outlined above.

5 This invention also relates to a recombinant DNA
vector comprising a replicon, Streptomyces gal operon, or
a functional and regulatable derivative thereof, and a
foreign functional DNA sequence operatively linked to such
operon. Such vector can be prepared by conventional
10 techniques. The replicon employed should be one known for
its ability to stably and extrachromosomally, maintain a
vector in the host organism which is to be the host
transformed with the vector.

15 This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, the Streptomyces gal
operon, or a functional and regulatable derivative
thereof, and a foreign functional DNA sequence operatively
linked to such operon; and to the method of preparing such
20 host which comprises transforming an appropriate host
microorganism with such vector. Appropriate host
microorganisms which may be employed in the method of this
invention include viruses, and eukaryotic and prokaryotic
cells or organisms, especially actinomycetes, such as
25 those of the genus Streptomyces. The most preferred host
microorganisms belong to the genus Streptomyces.

Preferred species of Streptomyces include Streptomyces
lividans, S. coelicolor, S. azureus and S. albus.

30 Transformation of such host microorganism with such vector
can be accomplished using conventional techniques such as
the method of Chater et al., Curr. Top. Micro. Imm., 96,
69-95 (1982). This invention also related to a method of
expressing the functional DNA sequence contained by such
transformed host of this invention which comprises
35 cultivating such transformed host under suitable
conditions such that the functional DNA sequence is

1 expressed. By "suitable conditions" is meant those
conditions which will allow the host to grow and which
enable the expression of the functional DNA sequence.
Such suitable conditions can be determined by one of skill
5 in the art using conventional techniques and will depend
on various factors, such as the host organism employed and
the functional DNA sequence to be expressed. This
invention is also related to a method of regulating the
expression of the functional DNA sequence contained by
10 such transformed host which comprises cultivating a
transformed host containing such functional DNA sequence
under appropriate conditions such that its expression is
regulatable. By "appropriate conditions" is meant those
conditions which enable the Streptomyces gal operon (and
15 thus the foreign functional DNA sequence) to be
regulatable. By "regulatable" is meant responsive to the
presence of galactose or its metabolites and the presence
of glucose or its metabolites in the growth media of the
transformed host cell. Such regulation can be carried out
20 by addition or deletion of galactose or glucose to the
transformed host's culture medium. The optimal levels of
galactose and/or glucose for up or down-regulation of the
expression of the foreign functional DNA coding sequence
by the transformed host of this invention can be readily
25 determined by one of skill in the art using conventional
techniques.

This invention also relates to a recombinant DNA
vector comprising a replicon, a Streptomyces gal operon P2
30 promoter expression unit, or a functional derivative
thereof, and a foreign functional DNA sequence operatively
linked to such unit. Such a vector can be prepared by
conventional techniques. The replicon employed should be
one known for its ability to stably, and extra-
chromosomally, maintain a vector in the host organism
35 which is to be transformed with the vector.

1 This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, the Streptomyces gal
operon P2 promoter expression unit, or a functional
5 derivative thereof, and a foreign functional DNA sequence
operatively linked to such unit; and to the method of
preparing such host which comprises transforming an
appropriate host microorganism with such vector. By the
term "operatively linked" is meant that a functional DNA
10 sequence is transcriptionally or translationally linked to
an expression control sequence (i.e., the Streptomyces gal
operon, P2 promoter expression unit, P1 promoter regulated
region, P1 promoter or P2 promoter) in such a way so that
the expression of the functional DNA sequence is under
15 control of the expression control sequence. Thus, for
example, a foreign functional DNA sequence can be
transcriptionally or translationally linked to the
Streptomyces gal operon by inserting such operon within
the Streptomyces gal operon P1 or P2 promoter transcript.
20 By the term "replicon" is meant that region of DNA on a
plasmid which functions to maintain, extrachromosomally,
such plasmid in a host microorganism or cell transformed
therewith. It has also been discovered that the
Streptomyces gal operon, and smaller portions thereof, is
25 useful as a nucleic acid probe to obtain homologous
sequences from other cells and organisms. Appropriate
host microorganisms which may be employed in the method of
this invention include any virus or eukaryotic or
prokaryotic cell or organism, especially any actinomycetes
30 such as those of the genus Streptomyces. The most
preferred host microorganisms belong to the genus
Streptomyces. Preferred species of Streptomyces include
Streptomyces lividans, S. coelicolor, S. azureus and S.
35 albus. Transformation of such host microorganism with
such vector can be accomplished using conventional

1 techniques such as the method of Chater et al., Curr. Top.
2 Micro. Imm., 96, 69-95 (1982). This invention also
3 related to a method of expressing the functional DNA
4 sequence contained by such transformed host of this
5 invention which comprises cultivating such transformed
6 host under suitable conditions such that the functional
7 DNA sequence is expressed. By "suitable conditions" is
8 meant those conditions which will allow the host to grow
9 and which enable the expression of the functional DNA
10 sequence. Such suitable conditions can be determined by
11 one of skill in the art using conventional techniques and
12 will depend on various factors, such as the host organism
13 employed and the functional DNA sequence to be expressed.

14 This invention also relates to a recombinant DNA
15 vector comprising a replicon, a Streptomyces gal operon P1
16 promoter regulated region, or a functional and regulatable
17 derivative thereof, and a foreign functional DNA sequence
18 operatively linked to such region. Such a vector can be
19 prepared by conventional techniques. The replicon
20 employed should be one known for its ability to stably and
21 extrachromosomally maintain a vector in the host organism
22 which is to be the host transformed with the vector.

23 This invention also relates to a transformed host
24 microorganism comprising a recombinant DNA vector wherein
25 said vector contains a replicon, a Streptomyces gal operon
26 P1 promoter regulated region, or a functional and
27 regulatable derivative thereof, and a foreign functional
28 DNA sequence operatively linked to such region; and to the
29 method of preparing such host which comprises transforming
30 an appropriate host microorganism with such vector.
31 Appropriate host microorganisms which may be employed
32 include any virus or eukaryotic or prokaryotic cell or
33 organism especially actinomycetes such as those of the
34 genus Streptomyces. The most preferred host
35 microorganisms belong to the genus Streptomyces.

1 Preferred species of Streptomyces include Streptomyces
2 lividans, S. coelicolor, S. azuraeus and S. albus.
3 Transformation of such host microorganism with such vector
4 can be accomplished using conventional techniques such as
5 the method of Chater et al., Curr. Top. Micro. Imm., 96,
6 69-95 (1982). This invention also related to a method of
7 expressing the foreign functional DNA sequence contained
8 by such transformed host of this invention which comprises
9 cultivating such transformed host under suitable
10 conditions such that the functional DNA sequence is
11 expressed. By "suitable conditions" is meant those
12 conditions which will allow the host to grow and which
13 enable the expression of the functional DNA sequence.
14 Such suitable conditions can be determined by one of skill
15 in the art using conventional techniques and will depend
16 on various factors, such as the host organism employed and
17 the functional DNA sequence to be expressed. This
18 invention also related to a method of regulating the
19 expression of the functional DNA sequence contained by
20 such transformed host which comprises cultivating a
21 transformed host containing such functional DNA sequence
22 under appropriate conditions such that its expression is
23 regulatable. By "appropriate conditions" is meant those
24 conditions which enable the Streptomyces gal operon P1
25 promoter regulated region (and thus the foreign functional
26 DNA sequence) to be regulatable. By "regulatable" is
27 meant responsive to the presence or absence of galactose
28 or its metabolites and the presence or absence of glucose
29 or its metabolites in the growth media of the transformed
30 host cell. Such regulation can be carried out by addition
31 or deletion of galactose or glucose to the transformed
32 host's culture medium.

33 This invention also relates to a recombinant DNA
34 vector comprising a replicon, a Streptomyces gal operon P2
35 promoter, or a functional derivative thereof, and a
foreign functional DNA sequence operatively linked to such

1 promoter. Such a vector can be prepared by conventional
techniques. The replicon employed should be one known for
its ability to stably and extrachromosomally maintain a
vector in the host organism which is to be the host
5 transformed with the vector.

This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, a Streptomyces gal operon
P2 promoter, or a functional derivative thereof, and a
10 foreign functional DNA sequence operatively linked to such
region; and to the method of preparing such host which
comprises transforming an appropriate host microorganism
with such vector. Appropriate host microorganisms which
may be employed include actinomycetes such as those of the
15 genus Streptomyces. The most preferred host
microorganisms belong to the genus Streptomyces.
Preferred species of Streptomyces include Streptomyces
lividans, S. coelicolor, S. azureus and S. albus.
Transformation of such host microorganism with such vector
20 can be accomplished using conventional techniques such as
the method of Chater et al., Curr. Top. Micro. Imm., 96,
69-95 (1982). This invention also related to a method of
expressing the foreign functional DNA sequence contained
by such transformed host of this invention which comprises
25 cultivating such transformed host under suitable
conditions such that the functional DNA sequence is
expressed. By "suitable conditions" is meant those
conditions which will allow the host to grow and which
enable the expression of the functional DNA sequence.
30 Such suitable conditions can be determined by one of skill
in the art using conventional techniques and will depend
on various factors, such as the host organism employed and
the functional DNA sequence to be expressed.

This invention also relates to a recombinant DNA
35 vector comprising a replicon, Streptomyces gal operon P1
promoter, or any regulatable and functional derivative

1 thereof, and a foreign functional DNA sequence operatively
linked to such region. Such a vector can be prepared by
conventional techniques. The replicon employed should be
one known for its ability to stably and extrachromosomally
5 maintain a vector in the host organism which is to be the
host transformed with the vector.

This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, the Streptomyces gal
10 operon P1 promoter, or any regulatable and functional
derivative thereof, and a foreign functional DNA sequence
operatively linked to such region; and to the method of
preparing such host which comprises transforming an
appropriate host microorganism with such vector.

15 Appropriate host microorganisms which may be employed
include viruses or prokaryotic or eukaryotic cells or
organisms, especially actinomycetes such as those of the
genus Streptomyces. The most preferred host
microorganisms belong to the genus Streptomyces.

20 Preferred species of Streptomyces include Streptomyces
lividans, S. coelicolor, S. azuraeus and S. albus.

Transformation of such host microorganism with such vector
can be accomplished using conventional techniques such as
the method of Chater et al., Curr. Top. Micro. Imm., 96,

25 69-95 (1982). This invention also relates to a method of
expressing the foreign functional DNA sequence contained
by such transformed host of this invention which comprises
cultivating such transformed host under suitable
conditions such that the functional DNA sequence is

30 expressed. By "suitable conditions" is meant those
conditions which will allow the host to grow and which
enable the expression of the functional DNA sequence.

Such suitable conditions can be determined by one of skill
in the art using conventional techniques and will depend
35 on various factors, such as the host organism employed and

1 the foreign functional DNA sequence to be expressed. This
invention also relates to a method of regulating the
expression of the functional DNA sequence contained by
such transformed host which comprises cultivating a
5 transformed host containing such foreign functional DNA
sequence under appropriate conditions such that its
expression is regulatable. By "appropriate conditions" is
meant those conditions which enable the gal operon P1
promoter (and thus the functional DNA sequence) to be
10 regulatable. By "regulatable" is meant responsive to the
presence or absence of galactose or its metabolites and
the presence of glucose or its metabolites in the growth
media of the transformed host cell. Such regulation can
be carried out by addition or deletion of galactose or
15 glucose to the transformed host's culture medium.

EXAMPLES

In the following Examples, specific embodiments
of the invention are more fully disclosed. These Examples
are intended to be illustrative of the subject invention
and should not be construed as limiting its scope. In all
20 Examples, temperature is in degrees Centigrade (°C).

By utilizing conventional methods, such as those
outlined in the following Examples, one of skill in the
art can isolate the gal operon from any galactose
utilizing strain of Streptomyces. Furthermore, by
25 utilizing techniques similar to those employed herein to
isolate the Streptomyces gal operon, one of skill in the
art can attempt to use the Streptomyces gal operon to
isolate a gal operon from other galactose utilizing other
strains of Streptomyces, especially S. coelicolor, S.
30 azuraeus, S. albus and other S. lividans strains.

Molecular genetic manipulations and other
techniques employed in the following Examples are
described in Hopwood et al., Genetic Manipulation of
35 Streptomyces: A Laboratory Manual, John Innes Foundation,
Norwich, England (1985).

1

ABBREVIATIONS

In the following Examples, the following abbreviations may be employed:

5

NaCl

MBSM (modified MBSM): See, Brawner et al., Gene, 40, 191 (1985) (in press)

10

MOPS: (3)-N-morpholino-(propane-sulfonic acid)

YEME + $MgCl_2$ + Glycine: [per liter(l)] 3 g yeast extract, 5 g peptone, 3 g malt extract, 10 g glucose, 10 g $MgCl_2 \cdot 6H_2O$, 340 g sucrose.

15

SL: Mix together $(NH_4)_2SO_4$ (1g/l); L-asparagine (2 g/l); K_2HPO_4 (9 g/l); NaH_2PO_4 (1 g/l) for 0.2% agar and autoclave. Then mix with yeast extract (20 g/l), $MgCl_2$ (5 g/l); $CuCl_2$ (0.1 g/l); Trace elements [20 ml/l - include $ZnCl_2$ -40 mg/l; $FeCl_3 \cdot 6H_2O$ (200 mg/l); $CuCl_2 \cdot 2H_2O$ (10 mg/l); $NaB_4O_7 \cdot 10H_2O$ (10 mg/l); $(NH_4)_6MO_7O_{24} \cdot 4H_2O$ (10 mg/l)] filter and sterilize.

20

YEME (Ym base): (per liter) yeast extract (3g); peptone (5g); malt extract (3g); $MgCl_2 \cdot 6H_2O$ (2g)

Ymglu: YEME + glucose (10g)

25

Ymgal: YEME + galactose (10g)

30

35

1

BACTERIAL STRAINS

In the following Examples, the following strains of E. coli are employed:

5

CGSC Strain #(a)	Strain Designation	Sex	Chromosomal Markers
4473 (<u>galE</u> ⁻)	W3109	F ⁻	<u>galE9</u> , ^(b) g ⁻ ; IN(rrnD-rrnE)1
4467 (<u>galT</u> ⁻)	W3101	F ⁻	<u>galT22</u> ^(b) g ⁻ ; IN(rrnD-rrnE)1
10 4498 (<u>galE</u> ⁻)	PL-2	Hfr	<u>thi-1</u> , <u>relA1</u> , <u>921E28</u> , g ⁻ , <u>spoT1</u>

(a) CGSC Strain # is the stock number designated for such strain by the E. coli Genetic Stock Center of the Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut, 06510, U.S.A.

15

(b) galE9 is the old Lederberg gal9; galT22 is the old Lederberg gal₁.

20

S1 ANALYSIS

S1 analysis is used to identify the 5' end of RNAs and the length of a RNA of interest. In the following Examples, S1 analysis refers to S1 experiments carried out according to the method of Weaver et al., Nucl. Acids Res., 7, 1175 (1979) and Berk et al., Proc. Natl. Acad. Sci. USA, 75, 1214 (1978).

25

EXAMPLE I

A. CLONING OF A STREPTOMYCES LIVIDANS GALACTOKINASE GENE.

30

Streptomyces lividans strain 1326 is described by Bibb et al., Mol. Gen. Genetics, 184, 230-240 (1981) and was obtained from D. A. Hopwood, John Innes Foundation, Norwich, England. Streptomyces lividans strain 1326 and S. lividans strain 1326 containing the pIJ6 plasmid were deposited in the Agricultural Research Culture Collection,

35

1 Peoria, Illinois, U.S.A., on June 1, 1982, under accession numbers NRRL 15091 and 15092, respectively.

5 High molecular weight chromosomal DNA was isolated from Streptomyces lividans strain 1326 according to the method of Maniatis et al., "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory (1982) and was size fractionated on a 10-40% sucrose gradient (See, Maniatis et al., cited above, p. 284-285).

10 Fractions of 18-24 kilobase (Kb) pairs were combined and dialyzed exhaustively against 10 mM Tris-HCl/1 mM EDTA (pH 8). Cosmid shuttle vector pJW357 was employed to clone such fractionated chromosomal DNA in its entirety. pJW357 was constructed by fusing pDPT6 cut with PstI to pIJ350 cut with PstI. pIJ350 is described in Kieser et al., Mol. Gen. Genet., 185, 223-238 (1982). pDPT6 is a tetracycline and chloramphenicol resistant, pBR322-based E. coli cosmid cloning vector described in Taylor et al., U.S. Patent No. 4,476,227. pJW357 has a unique EcoRI site in the
20 chloramphenicol resistance gene and a unique BamHI site in the Tc^R (tetracycline) resistance gene. pJW357 was digested with BamHI, dephosphorylated with alkaline phosphatase, and ligated to the fractionated chromosomal DNA described above.

25 The ligation product was packaged into bacteriophage heads (using the in vitro packaging system described by Maniatis et al., cited above, p. 264-265) and transfected into E. coli strain K21 which is a galK⁻ derivative of E. coli MM294. The transformation culture was grown for two hours in LB and for an additional two
30 hours in LB with 25 ug/ml chloramphenicol, washed three times with equal volumes of M9 media [see, Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory (1972)] without a carbon source, and plated
35 onto M9 agar [supplemented with proline, histidine, arginine, isoleucine, leucine, saline and .5% galactose;

1 See, Adams et al., Biochem. Biophys. Res. Comm., 89(2),
650-58 (1979)] with 30 mg/ml chloramphenicol. Twenty
plates were spread with approximately 200 transformants
per plate. After three days incubation at 37°C, no
5 transformants were detected. The minimal plates were then
sprayed with nicotinic acid to 5 ug/ml to supplement the
nicotinic acid requirement of E. coli strain K21, and the
incubation was continued for 3 more days at 37°C and for 2
additional days at room temperature. After such
10 incubation, the surviving colonies were patched to both
MacConkey galactose agar (MAC-GAL) [See, Miller et al.,
cited above] with 30 ug/ml chloramphenicol and to M63
minimal agar [See, Miller et al., cited above]
supplemented with .5% galactose, 5 ug/ml nicotinic acid, 5
15 ug/ml thiamine and 30 ug/ml chloramphenicol. Only two
colonies contained cosmid DNA that transformed E. coli K21
to a galK⁺ phenotype. Such cosmids were designated as
pSLIVGAL-1 and pSLIVGAL-2. Both colonies were light red
on MAC-GAL (i.e., they were galK⁺) and also grew on the
20 M63 medium.

Plasmids pSLIVGAL-1 and pSLIVGAL-2 were isolated
from the two galK⁺ colonies described above and were
transformed, according to the method of Chater et al.,
Curr. Top. Micro. Imm., 96, 69-95 (1982), into Streptomyces
25 lividans strain 1326-12K (a galK deficient strain isolated
after UV mutagenesis of S. lividans strain 1326, See,
Brawner et al., Gene, 40, 191 (1985), (in press). Plasmid
encoded complementation of the S. lividans 1326-12K
(galK⁻) host was tested by observing growth of spores
30 plated on MBSM-gal-thiostrepton according to the method of
Brawner et al., Gene, 40, 191 (1985) (in press).
pSLIVGAL-2 showed no detectable complementation of the
Streptomyces 1326-12K host.

Cell extracts were prepared from cultures grown
35 in SL medium supplemented with 1% glucose or galactose and
10)g/ml thiostrepton. The extracts were analyzed for

galactokinase production by immunoblot analysis (see, Brawner et al., Gene, 40, 191 (1985), in press) using rabbit antisera prepared against E. coli galactokinase.

The protein detected by immunoblot analysis was the approximate size of E. coli galK. Such protein appeared in galactose supplemented cultures of Streptomyces at levels several fold higher than in glucose cultures.

B. MAPPING OF THE S. LIVIDANS GALK REGION WITHIN A COSMID.

The galK region of the pSLIVGAL1 and pSLIVGAL2 cosmids, prepared as described above, was identified by cloning random fragments from the cosmids into a pUC18 derivative [See, Norrander et al., Gene, 26, 101-106 (1983)] and scoring complementation of E. coli strain MM294 (galK⁻) on MAC-GAL medium. The cosmid clone was partially digested with Sau3AI (using conditions which maximized the yield of 2 to 4 kilobase fragments), and the products of this reaction were ligated into the BglII site of pUC18-TT6, a derivative of pUC18 constructed by insertion of the following synthetic DNA sequence into the BamHI site of pUC18:

5'GATCAGATCTTGATCACTAGCTAGCTAG 3'

3' TCTAGAACTAGTGATCGATCGATCCTAG 5'

Twelve galK⁺ clones (red on MAC-GAL) were screened for size. One clone, designated as plasmid pSAU10, was the smallest and had an insert size of approximately 1.4 Kb.

In contrast to colonies containing pSLIVGAL1, the pUC clones were very red on MAC-GAL medium, indicating an increased production of galactokinase. The most likely explanation for the increased enzyme level was that the S. lividans galK gene was now being transcribed by an E. coli promoter which was stronger than the upstream promoter on the cosmid.

1

The insert of pSAU10 was isolated as an EcoRI to HindIII fragment (these sites flank the insert region of pUC18-TT6) for use as a probe for the S. lividans galK gene. The chromosomal DNA used in the cloning was restricted with EcoRI plus MluI and BamHI plus BglII, and then blotted according to the method of Southern, J. Mol. Biol., 98, 503 (1975). The pSAU10 fragment was nick translated and hybridized to the blot. The probe identified a 1.3 kb EcoRI-MluI fragment and a 5 kb BamHI-BglII fragment in the chromosomal digests. When this data was compared to the map of the cosmid insert, the location of the galK gene (between map positions 5 and 7, See Table A) was confirmed.

15

C. DNA SEQUENCING OF THE S. LIVIDANS GAL OPERON.

The Streptomyces lividans gal operon was sequenced by chain termination [(See, Sanger et al., Proc. Nat'l Acad. Sci., U.S.A., 74, 5463 (1977)) and chemical cleavage [See, Maxam and Gilbert, Methods in Enzymology, 65, 499 (1980)]. The initial sequences of galK were derived from Sau3AI and SalI fragments of the insert of pSAU6 (a 2.3 Kb sibling of pSAU10) shotgun cloned into the BamHI and SalI sites (respectively) of M13 mp 10 [See, Messing, Methods in Enzymology, 101, 20 (1983)]. Amino acid sequences of the S. lividans galT, galE and galK genes were predicted by computer, and further analyzed by comparison with amino acid sequences of the E. coli and or S. cerevisiae galactokinase, gal-1-phosphate uridyltransferase and UDP-4-epimerase enzymes. The sequences of these proteins were predicted by computer analysis using the total or partial DNA sequence of the genes which encode the gal enzymes [see, Debouck et al., Nuc Acids. Res., 13(6), 1841-1853 (1985), and Citron and Donelson, J. Bacteriology, 158, 269 (1984)]. Some homology was found between the inferred protein sequence

35

1

for the S. lividans galK, galT, galE gene products and
their respective E. coli and/or S. cerevisiae gene
products.

5

The complete DNA sequence of the S. lividans gal
operon is shown in Table 1. Included in Table 1 are the
transcription start sites for the operon's promoters and
the predicted amino acid sequences of the galT, galE and
galK gene products.

10

15

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-35-

TABLE 1
TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS
GALACTOSE OPERON

5

10

15

20

25

30

35

	-120	-110	-100	-90	-80	-70													
	CTA	CGC	CTC	CGC	GTT	CAG	TAA	TTG	AAC	ACT	TTT	GGT	GAT	GAA	CTT	TGT	TTG	ATT	GTG
		-60		-50		-40		-30		-20									
	ATG	TGA	CAG	GGG	GGT	GGT	GGG	TTG	TGA	TGT	GTT	ATG	TTT	GAT	TGT	GTT	CGA	TGA	TTG
																			galP1
	-10		1		10		20		30		40								
	ACG	GGC	GTC	CTG	GTG	ACT	CAT	GGG	TGG	GTG	CAG	AGG	AGT	GGC	GCA	GTG	AAG	AAG	ACC
					Met	Thr	His	Gly	Trp	Val	Gln	Arg	Ser	Ala	Ala	Val	Lys	Lys	Thr
					galT														
	50		60		70		80		90		100								
	TCG	ACC	CGG	CTG	GCC	GAC	GGC	CGT	GAG	CTG	GTC	TAC	TAC	GAC	CTG	CGC	GAC	GAC	ACC
	Ser	Thr	Arg	Leu	Ala	Asp	Gly	Arg	Glu	Leu	Val	Tyr	Tyr	Asp	Leu	Arg	Asp	Asp	Thr
		110		120		130		140		150									
	GTG	CGC	GAC	GCC	GTG	GAC	CGC	CGT	CCG	CTG	GAG	CGG	ACC	GTC	ACC	ACG	TCC	GAG	GTG
	Val	Arg	Asp	Ala	Val	Asp	Arg	Arg	Pro	Leu	Glu	Arg	Thr	Val	Thr	Thr	Ser	Glu	Val
	160		170		180		190		200		210								
	CGA	CGC	GAC	CCG	CTG	CTC	GGC	GAC	TCC	GGC	CCG	TCC	CGC	CTC	GCA	CCG	GCA	GGG	GGC
	Arg	Arg	Asp	Pro	Leu	Leu	Gly	Asp	Ser	Ala	Pro	Ser	Arg	Leu	Ala	Pro	Ala	Gly	Ala
	220		230		240		250		260		270								
	CAC	CTA	CCA	TCC	GCC	GGC	CGA	CCA	GTG	CCC	GCT	GTG	CCG	GTC	GGA	CGG	GGA	ACG	GCT
	His	Leu	Pro	Ser	Ala	Gly	Arg	Pro	Val	Pro	Ala	Val	Pro	Val	Gly	Arg	Gly	Thr	Ala
	280		290		300		310		320		330								
	GAG	CGA	GAT	CCG	GCC	TAT	GAC	GTG	GTG	GTC	TTC	GAC	AAT	CGC	TTT	CCC	TCC	CTG	GCC
	Glu	Arg	Asp	Pro	Ala	Tyr	Asp	Val	Val	Val	Phe	Glu	Asn	Arg	Phe	Pro	Ser	Leu	Ala

Table 1 - (cont'd)

-36-

			340			350			360			370			380				
5	GGT	GAC	TCC	GGG	CGC	TGC	GAG	GTC	GTC	TGC	TTC	ACC	TCC	GAC	CAC	GAC	GCC	TCC	TTC
	Gly	Asp	Ser	Gly	Arg	Cys	Glu	Val	Val	Cys	Phe	Thr	Ser	Asp	His	Asp	Ala	Ser	Phe
			390			400			410			420			430			440	
	GCC	GAC	CTG	AGC	GAG	GAG	CAG	GCC	CGG	CTG	GTC	GTC	GAC	GCC	TGG	ACG	GAC	CGC	ACC
	Ala	Asp	Leu	Ser	Glu	Glu	Gln	Ala	Arg	Leu	Val	Val	Asp	Ala	Trp	Thr	Asp	Arg	Thr
10			450			460			470			480			490			500	
	TCC	GAG	CTG	TCC	CAT	CTG	CCC	TCC	GTT	GAA	CAG	GTC	TTC	TGC	TTC	GAG	AAC	CGG	GGC
	Ser	Glu	Leu	Ser	His	Leu	Pro	Ser	Val	Glu	Gln	Val	Phe	Cys	<u>Phe</u>	<u>Glu</u>	<u>Asn</u>	<u>Arg</u>	<u>Gly</u>
			510			520			530			540			550				
15	GCC	GAG	ATC	GGG	GTG	ACG	CTG	GGT	CAC	CCG	CAC	GGG	CAG	ATC	TAC	GCC	TAC	CCG	TTC
	<u>Ala</u>	<u>Glu</u>	<u>Ile</u>	<u>Gly</u>	<u>Val</u>	<u>Thr</u>	<u>Leu</u>	<u>Gly</u>	<u>His</u>	<u>Pro</u>	<u>His</u>	<u>Gly</u>	<u>Gln</u>	<u>Ile</u>	<u>Tyr</u>	<u>Ala</u>	<u>Tyr</u>	<u>Pro</u>	<u>Phe</u>
			560			570			580			590			600			610	
	ACC	ACC	CCC	CGC	ACC	GCC	CTG	ATG	CTC	CGT	TCA	CTC	GCC	GCC	CAC	AAG	GAC	GCG	ACG
	Thr	Thr	Pro	Arg	Thr	Ala	Leu	Met	Leu	Arg	Ser	Leu	Ala	Ala	His	Lys	Asp	Ala	Thr
20			620			630			640			650			660			670	
	GGC	GGG	GGG	AAC	CTG	TTC	GAC	TCC	GTG	CTG	GAG	GAG	GAG	CTG	GCC	GGT	GAG	CGG	GTC
	Gly	Gly	Gly	Asn	Leu	Phe	Asp	Ser	Val	Leu	Glu	Glu	Glu	Leu	Ala	Gly	Glu	Arg	Val
			680			690			700			710			720				
25	GTC	CTG	GAG	GGT	GAG	CAC	TGG	GCC	GCC	TTC	GTC	GCG	TAC	GGC	GCG	CAC	TGG	CCG	TAC
	Val	Leu	Glu	Gly	Glu	His	Trp	Ala	Ala	Phe	Val	Ala	Tyr	Gly	Ala	His	Trp	Pro	Tyr
			730			740			750			760			770			780	
	GAG	GTG	CAC	CTC	TAC	CCG	AAG	CGG	CGG	GTG	CCC	GAT	CTG	CTC	GGG	CTC	GAC	GAG	GCG
	Glu	Val	His	Leu	Tyr	Pro	Lys	Arg	Arg	Val	Pro	Asp	Leu	Leu	Gly	Leu	Asp	Glu	Ala
30			790			800			810			820			830			840	
	GCT	CGC	ACA	GAA	TTC	CCC	AAG	GTC	TAC	CTG	GAG	CTG	CTG	AGG	CGT	TTC	GAC	CGG	ATC
	Ala	Arg	Thr	Glu	Phe	Pro	Lys	Val	Tyr	Leu	Glu	Leu	Leu	Arg	Arg	Phe	Asp	Arg	Ile

35

1 table 1 - (cont'd)

-37-

	850	860	870	880	890	900
5	TTC GGC GAG GGC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG GCG CCC TTC					
	Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe					
	910	920	930	940	950	
	GGC CAG CTG GAG TTC GAG GGT GTG ACG CCG GAC CAC TTC GCG CTC CAC CTG GAA CTT					
	Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu					
10	960	970	980	990	1000	1010
	TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CCG GCT CCG AAT CCG GCA					
	Phe Thr Ser Ala Val Arg Pro Ala Ser --- galP2					
	1020	1030	1040	1050	1060	1070
15	TGAACG TGTTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTGCGAGAG GTAGCCGAG					
	1080	1090	1100	1110	1120	1130
	TTC ATG AGC GGC AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC					
	Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val					
	galE					
20	1140	1150	1160	1170	1180	1190
	GCC CAG CAC TTG GTG GAG GCG GGC AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC					
	Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr					
	1200	1210	1220	1230	1240	
25	GGC TTC CGT GAG GTG TGC CCG CCG GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CGC					
	Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg					
	1250	1260	1270	1280	1290	1300
	CGC CAA GTG CGT GGA CCG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC					
	Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser					
30	1310	1320	1330	1340	1350	1360
	CAG GTC GGC GAG TCG GTC GTG AAC CCC GAG AAG TAC TGG GAC AAC AAC GTC GGT GGC					
	Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly					

35

1 Label 1 - (cont'd)

-38-

5 1370 1380 1390 1400 1410 1420
 ACC ATG GCG CTG CTG GAG GCC ATG CGG GCG GCG GTG CGG CGG CTC GTC TTC TCC
 Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

 1430 1440 1450 1460 1470
 TCC ACG GCC GCC ACG TAC GGC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
 Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

 10 1480 1490 1500 1510 1520 1530
 ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
 Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

 1540 1550 1560 1570 1580 1590
 15 GGC GAG GCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
 Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

 1600 1610 1620 1630 1640
 GGC GCG TAC GGG GAG TAC GGC GAG CGC CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
 Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

 20 1650 1660 1670 1680 1690 1700
 GTC CTT CAA GTG GCG CAG GGC AGG CGG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
 Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

 1710 1720 1730 1740 1750 1760
 25 CCG ACG CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCG AGG
 Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

 1770 1780 1790 1800 1810
 CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GCG GAG CAC CTC ATC TGC AAC CTG GGC
 Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

 30 1820 1830 1840 1850 1860 1870
 AAC GGC AAC GGC TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CGG CGG GTG ACG GGC
 Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

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1
Table 1 - (cont'd)

-39-

	1880	1890	1900	1910	1920	1930
5	CAT CCG ATC CCC GAG ATC ATG GCC CCG CCG CCG GCG CAC CCG CCG CTC CTC GTC					
	His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val					
	1940	1950	1960	1970	1980	1990
	GCG TCG GCC GCG ACC GCC CCG CAG AAG CTG GCG TGG AAC CCG TCG CCG CCG GAC CTC					
	Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu					
10	2000	2010	2020	2030	2040	
	GCC ATC GTG TCG GAC GCG TGG GAG TTG CCG CAG CCG CCG GCG GCG CAG TAG TA					
	Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---					
	2050	2060	2070	2080	2090	2100
15	ACC GCA GTT ACC GGA AAG GCG AGC GGT CAG GCG ATG GCC GAG GCT GTC GGG GAA CCG					
				Met Gly Glu Ala Val Gly Glu Pro		
	2110	2120	2130	2140	2150	
	TCG GCG AGC GGT TCG GGG AGC TGT ACG GGG CCG AGC CCG AGG GGG TGT GGG CCG CGA					
	Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg					
20	2160	2170	2180	2190	2200	2210
	GCG GCG CCG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GCG TTC GTC ATG					
	Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met					
	2220	2230	2240	2250	2260	2270
25	CCT TCG CCC TGC CCG ACC AGG TCG CCG CCG TCT CCC GCG CCG AAC GAC GCG ATC CTC					
	Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu					
	2280	2290	2300	2310	2320	
	GCG CTG CAC TCG GCC GAC GTC GAC GCC GAC CCG GTC GAG CTG CCG GTC GCC GAC CTG					
	Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu					
30	2330	2340	2350	2360	2370	2380
	GCC CCC GCG TCG GAC AAG TCC TGG ACG CCG TAC CCC TCG GCG GTC CTG TGG GCG CTG					
	Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu					

35

Table 1 - (cont'd)

-40-

	2390	2400	2410	2420	2430	2440
5	CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTG GCC TCG ACC GTC CCG Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr <u>Val</u> <u>Pro</u>					
	2450	2460	2470	2480	2490	
	TCC GGG GCG GGG CTC TCC TCC TCC GCG GCG CTG CAG GTC CGT CCC CTG GCG ATG AAC Ser <u>Gly</u> <u>Ala</u> <u>Gly</u> <u>Leu</u> <u>Ser</u> <u>Ser</u> <u>Ser</u> <u>Ala</u> Ala Leu Glu Val Arg Pro Leu Ala Met Asn					
10	2500	2510	2520	2530	2540	2550
	GAC CTG TAC GCC CTC GCG CTG CGC GGC TGG CAG CTG GCC CGG CTG TGC CAG CGC GCG Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala					
	2560	2570	2580	2590	2600	2610
15	GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACG GCG TCC GCC TGC Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys					
	2620	2630	2640	2650	2660	2670
	TGC GAG GCG GGC ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC AGC GGC AGA TCC Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser					
20	2680	2690	2700	2710	2720	
	CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GGG TCA ACC Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser					
	2730	2740	2750	2760	2770	2780
25	ACT CCC ACA GCG AGG GCG AGT ACG GCA AGC GCC GCG CGG GCT GCG AGA AGG GCG CCG Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro					
	2790	2800	2810	2820	2830	2840
	CGC TGC TGG GCG TCG ACG CGC TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu					
30	2850	2860	2870	2880	2890	
	GAG CCG CTG GGC GAC GAG GAG GAG GTG CCG CGC CTG GTC CGG CAC GTG GTG ACC GAG Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu					

35

1 Table 1 - (cont'd)

-41-

	2900	2910	2920	2930	2940	2950
5	GAC GAG CGC GTC GAA CGG GTG GTC GCG CTC CTC GAG TCG GCG ACA CCC GGC GCA TCG Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser					
	2960	2970	2980	2990	3000	3010
	GCG CCG TCC TGG TCG AGG GCC ACG CCT GCT GCG CGA CGA CTT CCG CAT CTC CTG CCG Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro					
10	3020	3030	3040	3050	3060	
	CGA GCT GGA CCT GGT CGT CGA CAC GGC CCT GGC CTC CGC GGC CCT CGG CGC CGG ATG Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg <u>Met</u>					
	3070	3080	3090	3100	3110	3120
15	ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTC GTG GAG GCC GCC GCG GTG GAC <u>Thr</u> <u>Gly</u> <u>Gly</u> <u>Gly</u> <u>Phe</u> <u>Gly</u> <u>Gly</u> Ser Ala <u>Ile</u> <u>Val</u> <u>Leu</u> <u>Val</u> Glu Ala Ala Ala Val Asp					
	3130	3140	3150	3160	3170	3180
	GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCC GCG GCG GGC CTC AAG CGT CCG CGG Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu <u>Lys</u> Arg Pro Arg					
20	3190	3200	3210	3220	3230	3240
	GTG TTC GAG GCG GTG CCT CGG CGG GGC GCG GCG CCT GGT CTG ACG GTC AGC CGA GCC Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala					
	3250	3260	3270	3280	3290	
25	GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CGG GGA TCA CGC ACA TGA Ala Ser Pro Ala Cys Thr Pro ---					
	3300					
	GCT GCT AGC CGC					

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EXAMPLE 2PROMOTERS OF THE S. LIVIDANS GAL OPERON

a) P1 promoter

(i) Summary

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This promoter is galactose inducible, glucose repressible and is the regulatable promoter for the entire Streptomyces gal operon. S1 data indicates that the Streptomyces lividans gal operon encodes a polycistronic transcript of approximately 3.4 kilobases (Kb). The transcript consists of approximately 1 Kb for galT, followed by approximately 1 Kb each for galE and galK. (See, Figure 1).

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15

Galactose induction of P1 is mediated, at least in part, by an operator sequence whose 5' end is located 31 bp upstream of the transcription start site and a repressor protein which recognizes the operator.

(ii) Experimental: Isolation, Localization, and Characterization of the P1 promoter.

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The sequences upstream of the Streptomyces lividans galK ATG were screened for promoters using the E. coli galK promoter probe system of Brawner, et al., Gene, 40, 191, (1985), in press. The HindIII-MluI fragment (See, Table A, map positions 1-5) was restricted with Sau3AI, ligated into the unique BamHI site of pK21 (Figure 2), and transformed into E. coli K21 (galK⁻) according to the method of Example 1. pK21 is a derivative of pSK03 and is an E. coli-Streptomyces shuttle vector containing the E. coli galK gene (See, Figure 2). The construction of pSK03 is described in Rosenberg et al., Genetic Engineering, 8, (1986), in press. The clones which expressed galK, i.e., those which had promoter activity, were identified on MacConkey - galactose plates. Two galK⁺ clones (designated as pK21 MH1 and 2) were transformed into Streptomyces 1326-12K (galK⁻).

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Extracts from transformants were cultured in Ymglu and Ymgal, and were analyzed by western blot analysis using anti-E. coli galactokinase antiserum. The blots showed significantly higher levels of galactokinase in the extracts from the galactose induced cultures.

5

pK21 MH1 and 2 were shown by restriction analysis to contain a 410 bp Sau3AI insert which is contained within the HindIII and BglII sites (see Table A, map positions 1-2) by Southern blot analysis according to the method of Southern, J. Mol. Biol., 98, 503 (1975). The cloned fragment was analyzed by S1 analysis using RNA isolated from Streptomyces lividans 1326-12K and E. coli K21 cultures. The fragment yielded a 290 nucleotide protected fragment after S1 digestion (indicating the 5' end of an mRNA 290 bp upstream of the Sau3AI site). Hybridization experiments (using single stranded M13 clones of this region) have identified the direction of transcription as left to right as shown in Figure 2 (i.e., transcription is going toward galK).

10

15

20

Conventional DNA sequence analysis and additional S1 mapping analysis were used to define the 5' end of the mRNA.

25

30

The sequences responsible for regulating galactose induction of P1 were localized by removing sequences upstream of the transcription start site by nuclease Bal31. Any change in promoter function or galactose induction by removal of these sequences was assessed using the E. coli galK promoter probe plasmid used to identify P1.

35

(iii) Construction of Gal Promoter Deletions.

Plasmid pHL5 was constructed by cloning a DNA fragment containing 100 bp of sequences downstream from the start of P1 transcription and 216 bp upstream from the start of P1 transcription into plasmid pUC19TT1. Plasmid pUC19TT1 is described in Norrander et al., Gene, 26, 101-106 (1983) and has the Unker as pUC18-TT6. See, Example IB. Deletions extending into the upstream

1 sequence preceeding P1 were generated by linearizing pHL5
with HindIII and treating the ends with nuclease Bal31.
The uneven ends were subsequently repaired with the Klenow
fragment of DNA polymerase I. Bal31-treated pHL5 was then
5 digested with BamHI and run on a 5% acrylamide gel. DNA
fragments in the molecular weight range of 100-300 bp were
eluted from the gel and subcloned into M13 mp 10 that had
been digested with HindII and BamHI. [See, Messing,
Methods in Enzymology, 101, 20 (1983)]. Individual
10 deletions were then sequenced from the single stranded
phage DNA the dideoxy chain termination method of Sanger,
et al., cited above.

(iv) Linking the P1 Promoter Deletions to the
E. coli galK Gene.

15 The various mp 10 clones were digested with BamHI
and HindIII. DNA fragments containing individual
deletions were isolated from low-melting point agarose
gels and then ligated to pK21 (see, Figure 2) that had
been digested with BamHI and HindIII. After
20 transformation into E. coli MM294, plasmid DNA was
isolated for each of the deletion derivatives and
transformed into Streptomyces Lividans 12K.

(v) Functional Assessment of Bal
31-Generated Deletions in S. lividans

25 For each individual promoter deletion, a single
thiostrepton resistant transformant was grown to late log
in YM base (YEME) + 10 ug/ml thiostrepton. Cells were
then pelleted, washed once in M56 media and resuspended in
M56 media (see Miller, et al., cited above). The washed
30 cells were then used to inoculate YM + 0.1M MOPS (pH 7.2)
+ 10 ug/ml thiostrepton supplemented with 1% galactose or
1% glucose. The cells were grown for 16 hours then
assayed for galactokinase activity.

Ten individual pK21 derivatives containing either
35 120, 67, 55, 34, 31, 24, 20, 18, 10 or 8 bp of sequence
upstream of the P1 transcription start site were analyzed

1

for galactokinase expression. These results showed that all the information necessary for galactose induction of P1, (i.e., 10-20 fold greater levels of galactokinase produced in galactose grown cells versus glucose grown cells) is included in the 31 bp of sequence upstream of P1. A deletion which leaves 34 bp of sequence upstream of P1 is partially inducible by galactose since galactose induced 6-fold greater amounts of galactokinase. Thus, one end of the operator must be situated within the sequences between the -24 and -31 position. The remaining deletions which leave either 20, 18, 10 or 8 bp of upstream sequence result in a constitutive P1 promoter, that is the levels of galactokinase produced were equivalent when cells were grown in the presence of galactose or glucose. Although the promoter deletions which retained 8 and 10 bp of P1 were constitutive, the amount of galactokinase produced was reduced 10 fold in comparison to the promoter deletions which retained 18 to 120 bp of upstream sequence. This result indicates that sequences between the -10 and -18 positions of -1 are essential for promoter function.

This data supports a model in which galactose induction of P1 is mediated, at least in part, by an operator sequence. One end of this sequence is 24 to 31 bp upstream of the P1 transcription start site. Removing part or all of the operator results in a promoter which is partially or totally derepressed. The other end of this sequence has not been defined by these experiments but it most likely is contained within the 24 to 31 bp of sequence upstream of the P1 transcription start site. In addition we cannot eliminate the possibility that the 3' end of the operator is also within the 100 bp downstream of the transcription start site since these sequences were contained within the smallest region needed to achieve galactose induction. These data also suggest that the factor which interacts with the operator sequence is a

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1 repressor protein. Finally, we do not have any evidence
which eliminates the possibility that P1 may be controlled
by factors other than a repressor (i.e., positive
5 activator such as lambda phage cII protein) to modulate
galactose induction promoter transcript.

b) P2 promoter

(i) Summary

10 The P2 promoter of the Streptomyces gal
operon is upstream of the galE gene and transcribes both
galE and galK genes.

P2 promoter expression is constitutive
(i.e., not glucose repressed/galactose induced) as shown
by S1 analysis.

15 (ii) Experimental: Isolation, Localization,
and Characterization of the P2 promoter.

The existence of the Streptomyces gal operon P2
promoter became apparent when the BglII-MluI fragment
(see, Table A, map positions 2-5) of S. lividans 1326 DNA
20 was inserted into plasmid pK21 (see, Figure 2) and
galactokinase expression was observed in Streptomyces
lividans 1326-12K transformed therewith.

DNA sequence analysis and S1 analysis were used
to identify the 5' end of the S. lividans gal operon P2.
25 The 5' end of the P2 promoter transcript is within 100 bp
upstream of the predicted galE ATG.

EXAMPLE 3

EVIDENCE OF A POLYCISTRONIC MESSAGE IN THE
30 STREPTOMYCES GAL OPERON

S1 analysis was used to map the transcripts
upstream and downstream of the Streptomyces lividans gal
operon galK gene. In general, overlapping DNA fragments
35 of 1-2 Kb were isolated from subclones, further
restricted, and end labelled. The message was followed
from the 3' end of galK to the upstream end at P1.

1

The 3' end of the Streptomyces lividans gal operon transcript probably occurs within the first hundred bases downstream of galK. Fragments 3' labelled at sites within the galK sequence were not protected to their full length (S1 analysis) if they extend into this downstream region. One experiment showed a possible protected region that terminated 50-100 bp downstream of the galK translation stop. The existence of a transcription terminator can be confirmed by conventional techniques by using a terminator probe system. The gal operon transcript clearly does not extend to the PvuII site (see, Table A, map position 8) because no full length protection of 5' labelled PvuII fragments occurs from that site.

15

5' end labelled fragments from two PvuII fragments, fragment I, (map positions 4-6, See, Table A), and fragment II, (map positions 6-8, See Table A), and the insert of pSaul0 were used as sources of probes for S1 walking from the 3' to 5' end of the message. All fragments through this region are protected, except the fragment containing the P2 promoter which shows partial and full protection. The complete protection from S1 digest indicates a polycistronic message which initiates upstream at P1 and continues to approximately 100 bp downstream of galK.

25

The above data is indirect evidence of a polycistronic mRNA of the Streptomyces gal operon. S1 analysis using a long contiguous DNA fragment (e.g., the 4.5 kb HindIII-SacI fragment, see map position 7 of Table A) has been used to confirm the transcript size.

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EXAMPLE 4

LOCALIZATION OF S. LIVIDANS GAL OPERON GALE AND GALT GENES

5 (i) Summary

The S. lividans gal operon galE gene was localized to 1.5 Kb PvuII fragment (map position, 4-6 of Table A) of pLIVGAL1 (Figure 1).

10 The S. lividans gal operon galE coding sequences extend through the MluI site (map position 5 of Table A).

The S. lividans gal operon galT gene was localized within the 1.15 Kb Nru-PvuII region (see, Table A, map positions 1a-4) of pSLIVGAL1.

15 The direction of S. lividans gal operon galE and galT transcription is the same as galK gene.

(ii) Experimental

It was necessary to identify the other functions contained on pLIVGAL1; specifically, does this plasmid encode for the enzyme galactose epimerase (galE) or the enzyme galactose transferase (galT). The Streptomyces gal operon galK gene was identified by its ability to complement an E. coli galK host. Thus, identification of the Streptomyces galT and galE genes was tested for by complementation of E. coli galE⁻ or galT⁻ hosts, respectively. An E. coli galT⁻ strain (CGSC strain #4467, W3101) and two galE⁻ strains (CGSC strain #4473; W3109 and CGSC strain #4498; PL-2) were obtained to test for complementation by the pSLIVGAL1 clone.

30 The ca. 9 Kb HindIII-SphI fragment (see, Table A, map positions 1-16) containing the Streptomyces lividans gal operon galK gene was inserted into pUC19. This fragment was situated within pUC19 such that transcription from the Plac promoter of pUC19 is in the same direction as the Streptomyces galK gene. pUC19 is described in Yanisch-Perrou, et al., Gene, 33, 103 (1983).
35 Complementation was assayed by growth on MacConkey-galactose plates. Cells which can utilize galactose

1 [galE⁺, galT⁺, galK⁺] will be red to pink on this
medium. E. coli strain PL-2 (see, Example 2) containing
pUC19 with the HindIII-SphI insert were pink on the
5 indicator plate indicating that the HindIII-SphI fragment
contains the Streptomyces lividans galE gene. The galE
gene was later mapped to within the 4.5 Kb HindIII-SacI
(the SacI site is near the region around map position 7-8
of Table A) fragment. If the sequences from the MluI site
10 (map position 5 of Table A) to the SacI site were removed
galE complementation of E. coli PL-2 was not detected.
The 5' end of the galK gene is 70 base pairs (bp) from the
MluI site. Therefore it seemed likely that the MluI site
was contained within the 5' or 3' end of the galE gene.
15 To determine the direction of galE transcription, the
HindIII-SacI fragment was inserted into pUC18. In this
configuration, the Streptomyces lividans galK gene is in
the opposite orientation with respect to Plac. The pUC18
HindIII-SphI clone did not complement E. coli PL-2
20 indicating the galE is transcribed in the same direction
as galK. In addition it was concluded that the MluI site
is contained within the 3' end of the galE gene. DNA
sequence analysis of the PvuII-MluI fragment (See, Table
A, map position 4-5) has identified an open reading frame
25 which encodes for a polypeptide of predicted molecular
weight of 33,000 daltons. The 5' end of this reading
frame is located approximately 176 bp from the PvuII site
(See, Table A, map position 4). Therefore, the sequencing
results support the conclusion that the 3' end of galE
30 traverses the MluI site (see, Table A, map position 5).

Similar experiments to localize the galT gene on
pSLIVGAL1 were attempted with the galT hosts.

The region between P1 and the 5' end of galE was
sequenced to identify the galT gene. Translation of the
DNA sequence to the amino acid sequence identified a
35 reading frame which encodes a protein showing a region of
homology to the yeast transferase.

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EXAMPLE 5

GALACTOSE INDUCTION OF S. LIVIDANS GAL OPERON GALK GENE

5

(i) Summary

Galactokinase expression is induced within one hour after the addition of galactose to culture medium.

Galactokinase expression is 10 times higher in the presence of galactose versus glucose or no additional carbon source within 6 hours after addition of the sugar.

10

(ii) Experimental

Galactose induction of the Streptomyces lividans galK gene was examined by assaying for galactokinase activity at 1, 3, 6 and 24 hours after the addition of galactose. Two liters of YM + 0.1M MOPS (pH 7.2) were inoculated with 2×10^7 spores of Streptomyces lividans 1326. After 21 hours growth, galactose or glucose were added to a final concentration of 1%. One, three, six and twenty four hours after the addition of sugar, cells were isolated and assayed for galactokinase activity. Total RNA was prepared by procedures described in Hopwood et al., cited above.

15

20

An increase in galactokinase synthesis was observed one hour after the addition of galactose. The increase continued over time (1 to 24 hours). S1 analysis of RNA isolated from the induced cultures confirmed that the increase in galK activity was due to increased levels of the P1 promoter transcript.

25

The S1 data and the induction studies suggest the following model for gene expression within the Streptomyces gal operon. The P1 promoter is the galactose inducible promoter. The P1 transcript includes galT, galE and galK. The P2 promoter is constitutive and its transcript includes galE and galK.

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It is interesting to note that the E. coli gal operon also has two promoters, P1 and P2. [See, Nusso et al., Cell, 12, 847 (1977)]. P1 is activated by cAMP-CRP binding whereas P2 is inhibited by cAMP-CRP. Translation of the E. coli gal operon galE coding sequence is more efficient when transcription initiates at P2 which serves to supply a constant source of epimerase even in the absence of galactose or the presence of glucose [See, Queen et al., Cell, 25, 241 (1981)]. The epimerase functions to convert galactose to glucose 1-phosphate during galactose utilization and convert UDP-glucose to UDP-galactose which is required for E. coli cell wall biosynthesis. It is possible that the P2 promoter of the Streptomyces galK operon also serves to supply epimerase and galactokinase in the absence of galactose or during secondary metabolism.

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EXAMPLE 6

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THE S. COELICOLOR GAL OPERON

(i) Summary

25

The restriction map of a fragment containing the S. coelicolor galK gene is identical to the restriction map of the S. lividans gal operon. (See, Figure 3).

S. coelicolor can grow on minimal media containing galactose as the sole carbon source.

Galactokinase expression in S. coelicolor is induced by the addition of galactose to the growth media.

30

A promoter analogous and most likely identical to P1 is responsible for galactose induction of the S. coelicolor gal operon.

(ii) Experimental

35

An approximately 14 kb partial Sau3A fragment containing the S. coelicolor galK gene was isolated by K. Kendall and J. Cullum at the University of Manchester Institute of Science and Technology, Manchester, UK

1 (unpublished data; personal communication). They were
able to localize the S. coelicolor galK gene within a 3 kb
EcoRI fragment by complementation of a S. coelicolor galK
mutant. The position of a number of restriction sites
5 within the S. lividans gal operon are identical to those
found within, upstream and downstream of the EcoRI
fragment containing the S. coelicolor galK gene
(Figure 3). Thus, it seems likely that the gene
organization of the S. coelicolor gal operon is identical
10 to the S. lividans gal operon.

Galactose induction of the S. coelicolor galK
gene was examined by immunoblotting. S. coelicolor was
grown in YM + 1% galactose or 1% glucose (Ymglu or Ymgal)
for 20 hours at 28 C. Galactokinase expression was
15 detected using rabbit antisera prepared against purified
E. coli galactokinase. The protein detected was the
approximate size of the E. coli and S. lividans galK gene
product. Galactokinase expression is galactose induced
since it was detected only when S. coelicolor was grown in
20 Ym + galactose (Ymgal).

S1 nuclease protection studies were performed to
determine if galactose induction of the S. coelicolor gal
operon is directed by a promoter analogous to the S.
lividans P1 promoter. RNA was isolated from S. coelicolor
25 grown in Ym + 1% galactose or 1% glucose (Ymgal or
Ymglu). The hybridization probe used for S1 analysis of
this RNA was a 410 bp Sau3A fragment which contains the S.
lividans P1 promoter, its transcription start site and the
5' end of the galT gene. The S1 protected fragment
30 detected by this analysis co-migrated with the protected
fragment detected when the probe was hybridized to RNA
isolated from S. lividans grown in the presence of
galactose. Thus, this result shows that galactose
induction of the S. coelicolor gal operon is directed by a
35 sequence indistinguishable from the S. lividans P1
promoter.

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It should be noted that the following strains of Streptomyces have been observed to be able to grow on medium containing galactose as the only carbon source:

5

S. albus J1074 (obtained from Dr. Chater, John Innes Foundation, Norwich, England)

S. carzinostaticus - ATCC accession number 15944

S. carzinostaticus - ATCC accession number 15945

10 S. antifibrinolyticus - ATCC accession number 21869

S. antifibrinolyticus - ATCC accession number 21870

S. antifibrinolyticus - ATCC accession number 21871

S. longisporus - ATCC accession number 23931

15

The abbreviation "ATCC" stands for the American Type Culture Collection, Rockville, Maryland, U.S.A.

20

While the above descriptions and Examples fully describe the invention and the preferred embodiments thereof, it is understood that the invention is not limited to the particular disclosed embodiments. Thus, the invention includes all embodiments coming within the scope of the following claims.

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Claims for the Contracting States :
BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

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1. A recombinant DNA molecule comprising a
Streptomyces gal operon or any regulatable and functional
derivative thereof.

10

2. The molecule of Claim 1 wherein the operon is
a S. lividans, S. coelicolor, S. azuraeus, S. albus, S.
carzinostaticus, S. antifibrinolyticus or S. longisporus
gal operon.

15

3. The molecule of Claim 2 wherein the operon is
a S. lividans gal operon.

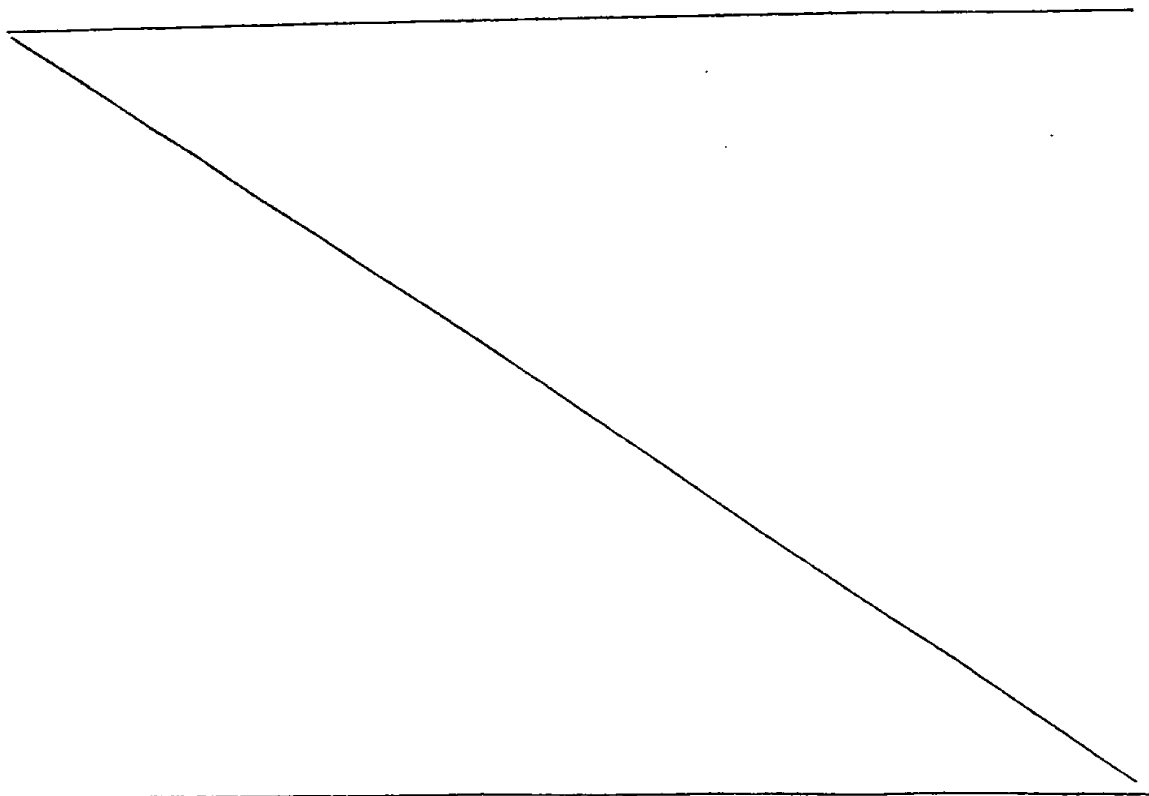
4. The molecule of Claim 3 which has the
following coding sequence:

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280 290 300 310 320 330
GAG CGA GAT CCG GCC TAT GAC GTG GTG GTC TTC GAG AAT CGC TTT CCC TCG CTG GCC
Glu Arg Asp Pro Ala Tyr Asp Val Val Val Phe Glu Asn Arg Phe Pro Ser Leu Ala

1

340 350 360 370 380
 5 GGT GAC TCC GCG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC
 Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe
 390 400 410 420 430 440
 GCC GAC CTG ACC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACG GAC CGC ACC
 Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr
 10 450 460 470 480 490 500
 TCC GAG CTG TCC CAT CTG CCC TCC GTT CAA CAG GTG TTC TGC TTC GAG AAC CGG GGC
 Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly
 510 520 530 540 550
 15 GCC GAG ATC GCG GTG ACG CTG GGT CAC CCG CAC GCG CAG ATC TAC GCC TAC CCG TTC
Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe
 560 570 580 590 600 610
 ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCC ACG
 Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr
 20 620 630 640 650 660 670
 GCG GCG GCG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC
 Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val
 680 690 700 710 720
 25 GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC CCG TAC GCG GCG CAC TGG CCG TAC
 Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr
 730 740 750 760 770 780
 GAG GTC CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GCG CTC GAC GAG GCG
 Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala
 30 790 800 810 820 830 840
 GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC
 Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile

35

5 850 860 870 880 890 900
 TTC GGC GAG GGC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG CCG CCG TTC
 Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe
 910 920 930 940 950
 GGG CAG CTG GAG TTC CAG GGT GTG ACG CGC GAC GAC TTC GCC CTC CAC CTG GAA CTT
 Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu
 10 960 970 980 990 1000 1010
 TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CGG GGT CCG AAT CCG GCA
 Phe Thr Ser Ala Val Arg Pro Ala Ser --- galP2
 1020 1030 1040 1050 1060 1070
 15 TGAACG TGTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTCGGAGAG GTAGCCAG
 1080 1090 1100 1110 1120 1130
 TTC ATG AGC GGG AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
 Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
 20 1140 1150 1160 1170 1180 1190
 GCC CAG CAC TTG GTG GAG GCG GCG AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
 Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr
 1200 1210 1220 1230 1240
 25 GGC TTC CGT GAG GTG TGC CCG CCG GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CCG
 Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg
 1250 1260 1270 1280 1290 1300
 CGC CAA GTG CGT GGA CCG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
 Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser
 30 1310 1320 1330 1340 1350 1360
 CAG GTC GCC GAG TCG GTC GTG AAG CCC GAG AAG TAC TGG GAC AAC AAC GTC GGT GGC
 Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly
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1370 1380 1390 1400 1410 1420
 ACC ATG GCG CTG CTG GAG GCC ATG CGG GCG GCG GGT GTG CCG CCG CTC GTC TTC TCC
 5 Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser
 1430 1440 1450 1460 1470
 TCC ACG GCC GCC ACG TAC GGC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCC CCG
 Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro
 10 1480 1490 1500 1510 1520 1530
 ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
 Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr
 1540 1550 1560 1570 1580 1590
 GGC GAG GCG GCG GCC CAC GGG CTG GCG GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
 15 Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala
 1600 1610 1620 1630 1640
 GGC GCG TAC GCG CAG TAC GGC GAG CGC CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
 Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu
 20 1650 1660 1670 1680 1690 1700
 GTC CTT CAA GTG GCG CAG GGC AGG CGG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
 Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr
 1710 1720 1730 1740 1750 1760
 CCG ACG CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCG AGG
 25 Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg
 1770 1780 1790 1800 1810
 CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GGC GAG CAC CTC ATC TGC AAC CTG GCG
 Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly
 30 1820 1830 1840 1850 1860 1870
 AAC GGC AAC GGC TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CCG CCG GTG ACG GCG
 Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

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1880 1890 1900 1910 1920 1930
 5 CAT CCC ATC CCC GAG ATC ATG GCC CGC CGC GGC CGC GAC CCC GCC GTC CTC GTC
 His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val
 1940 1950 1960 1970 1980 1990
 CGC TCG CCC GGC ACC GCC CGC GAG AAG CTC GGC TGG AAC CCC TCC CGC GCC GAC CTC
 Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu
 10 2000 2010 2020 2030 2040
 GCC ATC GTG TCG GAC GCG TCG GAG TTG CCC CAG CGC CGC GCC GCC CAG TAG TA
 Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---
 2050 2060 2070 2080 2090 2100
 15 ACC GCA GTT ACC GGA AAG GCG AGC GGT CAG GGC ATG GGC GAG CCT GTC GGG GAA CCG
 Met Gly Glu Ala Val Gly Glu Pro
 2110 2120 2130 2140 2150
 TCG GCC AGC GGT TCC GGG AGC TCT ACC GGG CGG AGC CGG AGG GGG TGT GGG CGC CGA
 Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg
 20 2160 2170 2180 2190 2200 2210
 GCG GCC CGG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GGC TTC GTC ATG
 Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met
 2220 2230 2240 2250 2260 2270
 25 CCT TCG CCC TCG CGC ACC AGC TCG CGC CGC TCT CCC GGC GCC AAC GAC GCC ATC CTC
 Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu
 2280 2290 2300 2310 2320
 CGC CTG CAC TCC GCC GAC GTC GAC GCC GAC CGC GTC GAG CTG CGC GTC GCC GAC CTC
 Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu
 30 2330 2340 2350 2360 2370 2380
 GCC CCC GCG TCG GAC AAG TCC TGG ACG GCG TAC CCC TCG GGC GTC CTG TGG GCG CTC
 Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu

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2390 2400 2410 2420 2430 2440
 5 CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTG GCC TCC ACC GTC CCG
 Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro
 2450 2460 2470 2480 2490
 10 TCC GCG GCG GCG CTC TCC TCC TCC GCG GCG CTG GAG GTC CGT CCC CTG GCG ATG AAC
 Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn
 2500 2510 2520 2530 2540 2550
 GAC CTG TAC GCC CTC CCG CTC CCG GCG TGG CAG CTG GCC CCG CTG TGC CAG CCG CCG
 Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala
 2560 2570 2580 2590 2600 2610
 15 GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACG GCG TCC GCC TGC
 Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys
 2620 2630 2640 2650 2660 2670
 20 TGC GAG GCG GCG ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC ACC GCG ACA TCC
 Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser
 2680 2690 2700 2710 2720
 CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGC TCG TCG ACA CCC GGG TCA AGC
 Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser
 2730 2740 2750 2760 2770 2780
 25 ACT CCC ACA GCG AGG GCG AGT ACG GCA AGC GCC GCG CCG GCT GCG AGA AGG GCG CCG
 Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Arg Arg Ala Pro
 2790 2800 2810 2820 2830 2840
 30 CCG TGC TGG GCG TCG ACG CCG TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG CCG CTG
 Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu
 2850 2860 2870 2880 2890
 GAG CCG CTG GCC GAC GAG GAG GAG GTG CCG CCG CTG GTC CCG CAC GTG GTG ACC GAG
 Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu

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2900 2910 2920 2930 2940 2950
 5 CAC GAG CGC GTC GAA CGG CTC GTC GCG CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG
 Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser
 2960 2970 2980 2990 3000 3010
 GCG CCG TCC TCG TCG AGG GCC ACG CCT GCT GCG CGA CGA CTT CCG CAT CTC CTC CCC
 Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro
 10 3020 3030 3040 3050 3060
 CGA GCT GGA CCT GGT CCT CGA CAC GGC CCT GCG CTC CGC GCG CCT CCG CGC CGG ATG
 Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met
 3070 3080 3090 3100 3110 3120
 15 ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG GAG GCC GCC GCG GTG CAC
 Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp
 3130 3140 3150 3160 3170 3180
 GCC GTC ACC AAG GCG GTC GAG CAC GCC TTC GCG GCG GCG GCG CTC AAG CGT CCG CGG
 Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg
 20 3190 3200 3210 3220 3230 3240
 GTG TTC GAG GCG GTG CCT CGG CGG GCG GCG GCG CCT GGT CTG ACG GTC ACC CGA GCC
 Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala
 3250 3260 3270 3280 3290
 25 GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CCG GGA TCA CCG ACA TGA
 Ala Ser Pro Ala Cys Thr Pro ---
 3300
 GCT GCT AGC CGC

30

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1 5. The molecule of Claim 1 which further
comprises a foreign functional DNA sequence operatively
linked to such operon.

5 6. A transformed host microorganism or cell
comprising the molecule of Claim 5.

7. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 5
which comprises transforming an appropriate host
microorganism or cell with such molecule.

10 8. A recombinant DNA vector comprising the
molecule of Claim 5, and, optionally, additionally
comprising a replicon.

9. A transformed host microorganism or cell
comprising the recombinant DNA vector of Claim 8.

15 10. A method of preparing a transformed host
microorganism or cell comprising the recombinant DNA
vector of Claim 8 which comprises transforming an
appropriate host microorganism or cell with such vector.

20 11. A method of expressing a foreign functional
DNA sequence which comprises cultivating a transformed
host microorganism or cell comprising the recombinant DNA
vector of Claim 8 under suitable conditions such that the
functional DNA sequence is expressed.

25 12. A method of regulating the expression of a
foreign functional DNA sequence which comprises
cultivating a transformed host microorganism or cell which
contains the recombinant DNA vector of Claim 8 under
appropriate conditions such that expression of the
sequence is regulatable.

30 13. A recombinant DNA molecule comprising a
Streptomyces gal operon P2 promoter expression unit or any
functional derivative thereof.

35 14. The molecule of Claim 13 wherein the
expression unit is a S. lividans, S. coelicolor, S.
azuraeus, S. albus, S. carzinostaticus, S.
antifibrinolyticus or S. longisporus gal operon P2
promoter expression unit.

1. The molecule of Claim 14 which is a S. lividans gal operon P2 promoter expression unit.

16. The molecule of Claim 13 which further comprises a foreign functional DNA sequence operatively linked to such expression unit.

17. A transformed host microorganism or cell comprising a recombinant DNA molecule wherein such molecule comprises the molecule of Claim 16.

18. A method of preparing a transformed host microorganism comprising the molecule of Claim 16 which comprises transforming an appropriate host microorganism or cell with such molecule.

19. A recombinant DNA vector comprising the molecule of Claim 16, and, optionally, additionally comprising a replicon.

20. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19.

21. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19 which comprises transforming an appropriate host microorganism with such vector.

22. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19 under suitable conditions such that the functional DNA sequence is expressed.

23. A recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof.

24. The molecule of Claim 23 wherein the region is a S. lividans, S. coelicolor, S. azureus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon P1 promoter regulated region.

25. The molecule of Claim 24 wherein the region is a S. lividans gal operon P1 promoter regulated region.

1 26. The molecule of Claim 23 which further
comprises a foreign functional DNA sequence operatively
linked to such regulated region.

5 27. A transformed host microorganism or cell
comprising the molecule of Claim 26.

28. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 26
which comprises transforming an appropriate host
microorganism or cell with such molecule.

10 29. A recombinant DNA vector comprising the
molecule Claim 26, and, optionally, additionally
comprising a replicon.

30. A transformed host microorganism or cell
comprising a recombinant DNA vector of Claim 29.

15 31. A method of preparing a transformed host
microorganism or cell comprising the recombinant DNA
vector of Claim 29 which comprises transforming an
appropriate host microorganism or cell with such vector.

20 32. A method of expressing a foreign functional
DNA sequence which comprises cultivating a transformed
host microorganism or cell comprising the recombinant DNA
vector of Claim 29 under suitable conditions such that the
functional DNA sequence is expressed.

25 33. A method of regulating the expression of a
foreign functional DNA sequence which comprises
cultivating a transformed host microorganism or cell which
contains the recombinant DNA vector of Claim 29 under
appropriate conditions such that expression of the
sequence is regulatable.

30 34. A recombinant DNA molecule comprising a
Streptomyces gal operon P2 promoter or any functional
derivative thereof.

35 35. The molecule of Claim 34 wherein the promoter
is a S. lividans, S. coelicolor, S. azureus, S. albus, S.
carzinostaticus, S. antifibrinolyticus or S. longisporus
gal operon P2 promoter.

1

36. The molecule of Claim 35 wherein the promoter is a S. lividans gal operon P2 promoter.

5

37. The molecule of Claim 34 which further comprises a foreign functional DNA sequence operatively linked to the P2 promoter.

38. A transformed host microorganism or cell comprising the molecule of Claim 37.

10

39. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 37 which comprises transforming an appropriate host microorganism or cell with such molecule.

40. A recombinant DNA vector comprising the molecule of Claim 37 and, optionally, additionally comprising a replicon.

15

41. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40.

20

42. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40 which comprises transforming an appropriate host microorganism with such vector.

25

43. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40 under suitable conditions such that the functional DNA sequence is expressed.

44. A recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter or any regulatable and functional derivative thereof.

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45. The molecule of Claim 44 wherein the promoter is a S. lividans, S. coelicolor, S. azureus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon P1 promoter.

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46. The molecule of Claim 45 wherein the promoter is a S. lividans gal operon P1 promoter.

47. The molecule of Claim 44 which further comprises a foreign functional DNA sequence operatively linked to the P1 promoter.

1 48. A transformed host microorganism or cell
comprising the molecule of Claim 47.

5 49. A method of preparing a transformed host
microorganism or cell comprising molecule of Claim 47
which comprises transforming an appropriate host
microorganism or cell with such molecule.

50. A recombinant DNA vector comprising the
molecule of Claim 47, and, optionally, additionally
comprising a replicon.

10 51. A transformed host microorganism or cell
comprising the recombinant DNA vector of Claim 50.

52. A method of preparing a transformed host
microorganism or cell comprising the recombinant DNA
vector of claim 50 which comprises transforming an
15 appropriate host microorganism with such vector.

53. A method of expressing a foreign functional
DNA sequence which comprises cultivating a transformed
host microorganism or cell comprising the recombinant DNA
vector of Claim 50 under suitable conditions such that the
20 functional DNA sequence is expressed.

54. A method of regulating the expression of a
foreign functional DNA sequence which comprises
cultivating a transformed host microorganism or cell which
contains the recombinant DNA vector of Claim 50 under
25 appropriate conditions such that expression of the
sequence is regulatable.

55. A recombinant DNA molecule comprising a
Streptomyces gal operon galE gene, or any functional
derivative thereof.

30 56. The molecule of Claim 55 wherein the gene is
a S. lividans, S. coelicolor, S. azuraeus, S. albus, S.
carzinostaticus, S. antifibrinolyticus or S. longisporus
gal operon galE gene.

35 57. The molecule of Claim 56 wherein the gene is
a S. lividans gal operon galE gene.

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58. The molecule of Claim 55 which further comprises a foreign functional DNA sequence operatively linked to the galE gene.

5

59. A transformed host microorganism or cell comprising the molecule of Claim 58.

10

60. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 58 which comprises transforming an appropriate host microorganism or cell with such molecule.

61. A recombinant DNA molecule comprising a Streptomyces gal operon galT gene, or any functional derivative thereof.

15

62. The molecule of Claim 61 wherein the gene is a S. lividans, S. coelicolor, S. azuraeus or S. albus, S. carzinostaticus, S. antifibrinolyticus and S. longisporus gal operon galT gene.

63. The molecule of Claim 62 wherein the gene is a S. lividans gal operon galT gene.

20

64. The molecule of Claim 61 which further comprises a foreign functional DNA sequence operatively linked to the galT gene.

65. A transformed host microorganism or cell comprising the molecule of Claim 64.

25

66. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 64 which comprises transforming an appropriate host microorganism or cell with such molecule.

30

67. A recombinant DNA molecule comprising a Streptomyces lividans gal operon galK gene, or any functional derivative thereof.

68. The molecule of Claim 67 wherein the gene is a S. lividans, S. coelicolor, S. azuraeus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon galK gene.

35

69. The molecule of Claim 68 wherein is a S. lividans gal operon galK gene.

1 70. The molecule of Claim 67 which further
comprises a foreign functional DNA sequence operatively
linked to the galK gene.

5 71. A transformed host microorganism or cell
comprising the molecule of Claim 70.

72. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 70
which comprises transforming an appropriate host
microorganism or cell with such molecule.

10 73. A method of enabling a nongalactose
utilizing host microorganism or cell to utilize galactose
which comprises transforming such host with a recombinant
DNA vector or molecule comprising a Streptomyces gal
operon, or any portion of the Streptomyces gal operon
15 which is adequate to enable such transformed host to
utilize galactose, or any functional derivative thereof.

74. A transformed host prepared by the method of
Claim 73.

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Claims for the Contracting States : AT, ES, GR

1. A method of preparing a transformed host microorganism or cell comprising the molecule which has 5 the following sequence :

```

10      -120      -110      -100      -90      -80      -70
      .         .         .         .         .         .
CTA CGC CTC CGC GTT CAG TAA TTG AAC ACT TTT GGT GAT GAA CTT TGT TTG ATT GTC

      -60      -50      -40      -30      -20
      .         .         .         .         .
ATG TGA CAG GGG GGT GGT GGC TTG TGA TGT GTT ATG TTT GAT TGT GTT GGA TGA TTG
                                     galPl

15      -10      1      10      20      30      40
      .         .         .         .         .         .
ACG GGC GTC CTG GTG ACT CAT GGG TGG GTG CAG AGG AGT GCG GCA GTG AAG AAG ACC
      Met Thr His Gly Trp Val Gln Arg Ser Ala Ala Val Lys Lys Thr
      .         .         .         .         .         .
      50      60      70      80      90      100
      .         .         .         .         .         .
20      TCG ACC CGG CTG GCC GAC GGC CGT GAG CTG GTC TAC TAC GAC CTG CGC GAC GAC ACC
      Ser Thr Arg Leu Ala Asp Gly Arg Glu Leu Val Tyr Tyr Asp Leu Arg Asp Asp Thr
      .         .         .         .         .         .
      110      120      130      140      150
      .         .         .         .         .
GTG CGC GAC GCC GTG GAC CGC CGT CCG CTG GAG CGG ACC GTC ACC ACC TCC GAC GTG
      Val Arg Asp Ala Val Asp Arg Arg Pro Leu Glu Arg Thr Val Thr Thr Ser Glu Val

25      160      170      180      190      200      210
      .         .         .         .         .         .
CGA CGC GAC CCG CTG CTC GGC GAC TCC GCG CCG TCG CGC CTC GCA CCG GCA GGC GCG
      Arg Arg Asp Pro Leu Leu Gly Asp Ser Ala Pro Ser Arg Leu Ala Pro Ala Gly Ala
      .         .         .         .         .         .
      220      230      240      250      260      270
      .         .         .         .         .         .
CAC CTA CCA TCC GCC GGC CGA CCA GTG CCC GCT GTG CCG GTC GGA CGG GGA ACC GCT
      His Leu Pro Ser Ala Gly Arg Pro Val Pro Ala Val Pro Val Gly Arg Gly Thr Ala

30      280      290      300      310      320      330
      .         .         .         .         .         .
GAG CGA GAT CCG GCC TAT GAC GTG GTG GTC TTC GAG AAT CGC TTT CCC TCG CTG GCC
      Glu Arg Asp Pro Ala Tyr Asp Val Val Val Phe Glu Asn Arg Phe Pro Ser Leu Ala

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35

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340 350 360 370 380
 5 GCT GAC TCC GGG CGC TGC GAG GTC GTC TCC TTC ACC TCC GAC CAC GAC GCC TCC TTC
 Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe
 390 400 410 420 430 440
 GCC GAC CTG ACC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACC GAC CGC ACC
 Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr
 10 450 460 470 480 490 500
 TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTC TTC TGC TTC GAG AAC CGG GGC
 Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly
 510 520 530 540 550
 15 GCC GAG ATC CGG CTG ACC CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC
 Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe
 560 570 580 590 600 610
 ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACC
 Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr
 20 620 630 640 650 660 670
 GGC GGC GGC AAC CTG TTC GAC TCC GTC CTG GAG GAG GAG CTG GCC GGT GAG CCG GTC
 Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val
 680 690 700 710 720
 25 GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC
 Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr
 730 740 750 760 770 780
 GAG GTG CAC CTC TAC CCG AAG CGG CCG GTG CCC GAT CTG CTC GCG CTC GAC GAG GCG
 Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala
 30 790 800 810 820 830 840
 GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CCG ATC
 Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile

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1

5 850 860 870 880 890 900
TTC GGC GAG GCC GAG CCC CCG ACC CCC TAC ATC GCG GCC TCG CAC CAG GCG CCG TTC
Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe

 910 920 930 940 950
CGC CAG CTG GAG TTC GAG GGT GTG ACC CCG GAC GAC TTC GCG CTC CAC CTG GAA CTT
Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu

10 960 970 980 990 1000 1010
TTC ACT TCC GCC GTA CGT CCG GCA ACC TGA AGT TCC TCG CCG GCT CCG AAT CCG GCA
Phe Thr Ser Ala Val Arg Pro Ala Ser --- galP2

 1020 1030 1040 1050 1060 1070
15 TGAACC TGTTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTCGGAGAG GTAGCGAG

1080 1090 1100 1110 1120 1130
TTC ATG AGC GGC AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
gale

20 1140 1150 1160 1170 1180 1190
GCC CAG CAC TTG GTG GAG GCG GGG AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr

 1200 1210 1220 1230 1240
25 GGC TTC CGT GAG GTG TGC CCG CCG GTG CCT CGT TCG TCG AGC CGA CAT CCG GGA CCG
Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg

1250 1260 1270 1280 1290 1300
CGC CAA GTG CGT GGA CCG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser

30 1310 1320 1330 1340 1350 1360
CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TCG GAC AAC AAC GTC GGT GGC
Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly

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5 1370 1380 1390 1400 1410 1420
 ACC ATG GCG CTC CTG GAG GCC ATG CCG GCG GCG GGT GTG CCG CCG CTC GTC TTC TCC
 Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

 1430 1440 1450 1460 1470
 TCC ACC GCC GCC ACC TAC GGC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
 Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

10 1480 1490 1500 1510 1520 1530
 ACC AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
 Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

 1540 1550 1560 1570 1580 1590
 GGC GAG GCG GCG GCC CAC GGC CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC CCG
 Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

15 1600 1610 1620 1630 1640
 GGC GCG TAC GGC GAG TAC GGC GAG CCG CAC GAG CCC GAG TCG CAT CTG ATT CCG CTG
 Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

20 1650 1660 1670 1680 1690 1700
 GTC CTT CAA GTG GCG CAG GGC AGG CCG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
 Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

 1710 1720 1730 1740 1750 1760
 CCG ACC CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACC TCG CCG ACC TGG CCG AGG
 Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

25 1770 1780 1790 1800 1810
 CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GCG GAG CAC CTC ATC TGC AAC CTG GCG
 Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

30 1820 1830 1840 1850 1860 1870
 AAC GGC AAC GGC TTC TCC GTC CCG GAG GTC GTC GAG ACC GTG CCG CCG GTG ACC GCG
 Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

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5 2390 2400 2410 2420 2430 2440
 CGC GAG GCC GGA CAC GAG CTG ACC GCC GCC GAC GTC CAC CTC GCC TCG ACC GTC CCG
 Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro

 2450 2460 2470 2480 2490
 TCC GCG GCG GGG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC
 Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn

10 2500 2510 2520 2530 2540 2550
 GAC CTG TAC GCC CTC GCG CTG CCG GCG TGG CAG CTG GCC CCG CTG TGC CAG CCG GCG
 Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala

 2560 2570 2580 2590 2600 2610
 GAG AAC GTC TAC GTC GCG GCC CCC GTC GCG ATC ATG GAC CAG ACC GCG TCC GCC TGC
 Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys

15 2620 2630 2640 2650 2660 2670
 TGC GAG GCG GCG ACC CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC ACC GCG AGA TCC
 Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser

20 2680 2690 2700 2710 2720
 CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GCG TCA AGC
 Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser

 2730 2740 2750 2760 2770 2780
 ACT CCC ACA GCG AGG GCG ACT ACC GCA AGC GCC GCG CCG GCT GCG AGA AGG GCG CCG
 Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro

25 2790 2800 2810 2820 2830 2840
 CCG TGC TGG GCG TCG ACC CCG TGC GAC GTG CCG TAC GCC GAC CTC GAC GCG GCG CTG
 Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu

30 2850 2860 2870 2880 2890
 GAG CCG CTG GCG GAC GAG GAG GAG GTG CCG CCG CTG GTC CCG CAC GTC GTC ACC GAG
 Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu

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2900 2910 2920 2930 2940 2950
 5 GAC GAG CGC GTC GAA CGC CTC GTC GCG CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG
 Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser
 2960 2970 2980 2990 3000 3010
 GCG CCG TCC TCG TCC AGG GCG ACC CCT GCT GCG CGA CGA CTT CCG CAT CTC CTG CCC
 Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro
 10 3020 3030 3040 3050 3060
 CGA GCT GGA CCT GGT CCT CGA CAC GGC CCT GCG CTC CCG GCG CCT CCG CCG CCG ATG
 Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met
 3070 3080 3090 3100 3110 3120
 15 ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG GAG GCG GCG CCG GTG GAC
 Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp
 3130 3140 3150 3160 3170 3180
 GCG GTC ACC AAG GCG GTC GAG GAC GCG TTC GCG GCG GCG GCG CTC AAG CGT CCG CCG
 Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg
 20 3190 3200 3210 3220 3230 3240
 GTG TTC GAG GCG GTG CCT CCG CCG GCG GCG GCG CCT GGT CTG ACC GTC ACC CGA GCG
 Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala
 3250 3260 3270 3280 3290
 25 GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CCG GGA TCA CCG ACA TGA
 Ala Ser Pro Ala Cys Thr Pro ---
 3300
 GCT GCT ACC CCG

30

which comprises transforming an appropriate host micro-
organism or cell with such molecule.

35

2. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the sequence of Claim 1 which comprises transforming an appropriate host microorganism or cell
5 with such vector.

3. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 2 under suitable conditions such
10 that the functional DNA sequence is expressed.

4. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains the recombinant DNA vector of Claim 2 under
15 appropriate conditions such that expression of the sequence is regulatable.

5. A method of preparing a transformed host microorganism comprising a recombinant DNA molecule comprising a Streptomyces gal operon P2 promoter
20 expression unit or any functional derivative thereof and a foreign functional DNA sequence operatively linked to such expression unit, which comprises transforming an appropriate host microorganism or cell with such molecule.

25 6. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 5 and, optionally, additionally comprising a replicon which comprises transforming an appropriate host microorganism with
30 such vector.

7. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 5 and, optionally,
35 additionally comprising a replicon, under suitable con-

ditions such that the functional DNA sequence is expressed.

8. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof and a foreign functional DNA sequence operatively linked to such regulated region, which comprises transforming an appropriate host micro-
10 organism or cell with such molecule.

9. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 8 and, optionally, additionally comprising a replicon which comprises
15 transforming an appropriate host microorganism or cell with such vector.

10. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant
20 DNA vector of Claim 8 and, optionally, additionally comprising a replicon under suitable conditions such that the functional DNA sequence is expressed.

11. A method of regulating the expression of a foreign functional DNA sequence which comprises culti-
25 vating a transformed host microorganism or cell which contains a recombinant DNA vector comprising the molecule of Claim 8 and, optionally, additionally comprising a replicon, under appropriate conditions such that expression of the sequence is regulatable.

30 12. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon P2 promoter or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the P2

promoter, which comprises transforming an appropriate host microorganism or cell with such molecule.

13. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 12 and, optionally, additionally comprising a replicon, which comprises transforming an appropriate host microorganism with such vector.

14. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising a recombinant DNA molecule of Claim 12 and, optionally, additionally comprising a replicon, under suitable conditions such that the functional DNA sequence is expressed.

15. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter or any regulatable and functional DNA sequence operatively linked to the P1 promoter, which comprises transforming an appropriate host microorganism or cell with such molecule.

16. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, which comprises transforming an appropriate host microorganism with such vector.

17. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, under suitable conditions such that the functional DNA sequence is expressed.

18. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, under appropriate conditions such that expression of the sequence is regulatable.

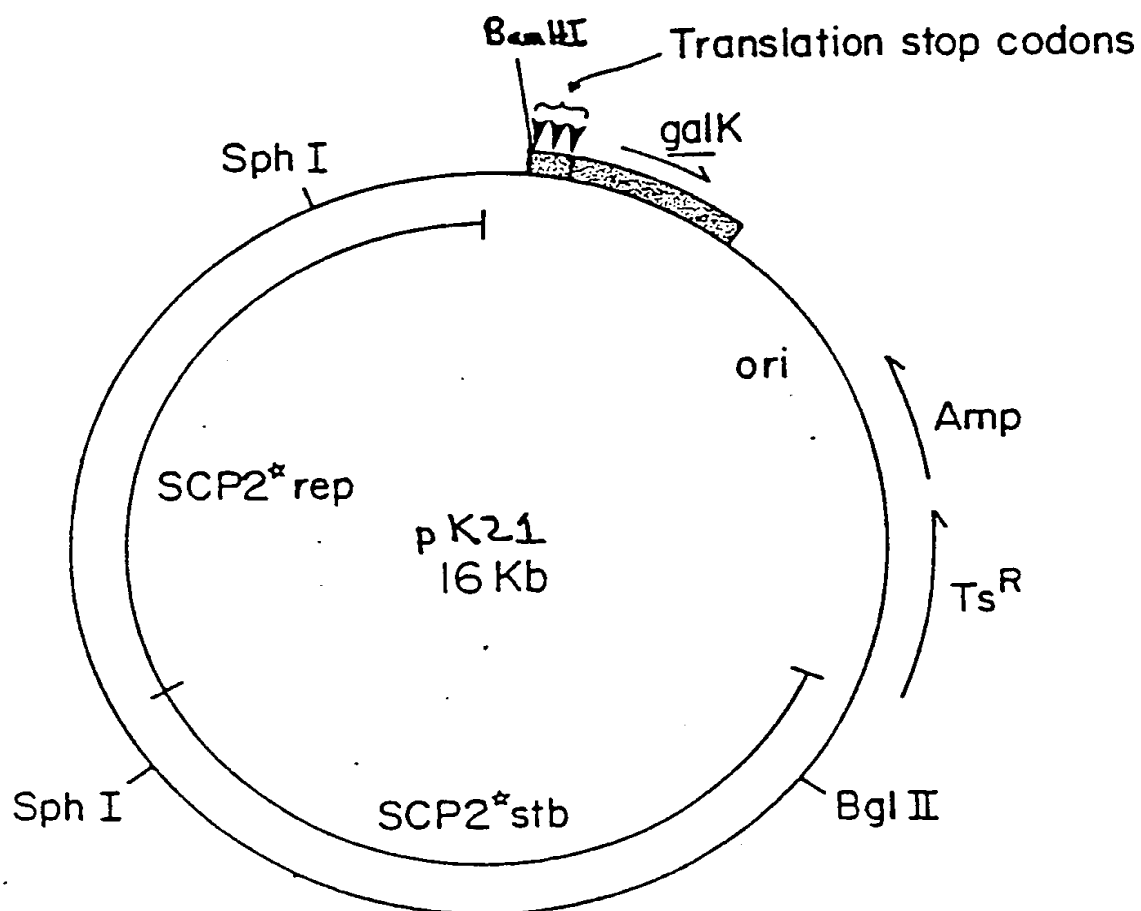
19. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon galE gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galE gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

20. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon galT gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galT gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

21. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces lividans gal operon galK gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galK gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

22. A method of enabling a nongalactose utilizing host microorganism or cell to utilize galactose which comprises transforming such host with a recombinant DNA vector or molecule comprising Streptomyces gal operon, or any portion of the Streptomyces gal operon which is adequate to enable such transformed host to utilize galactose, or any functional derivative thereof.

Figure 1



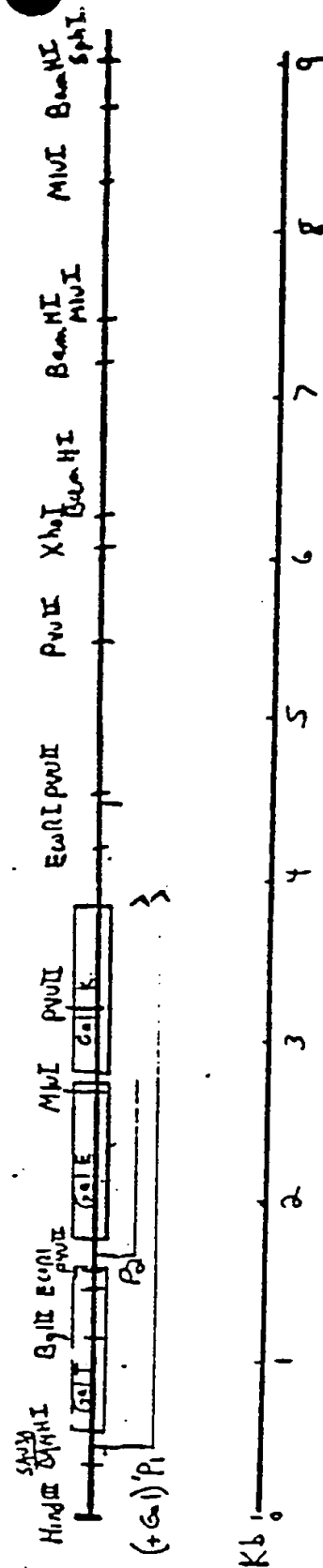
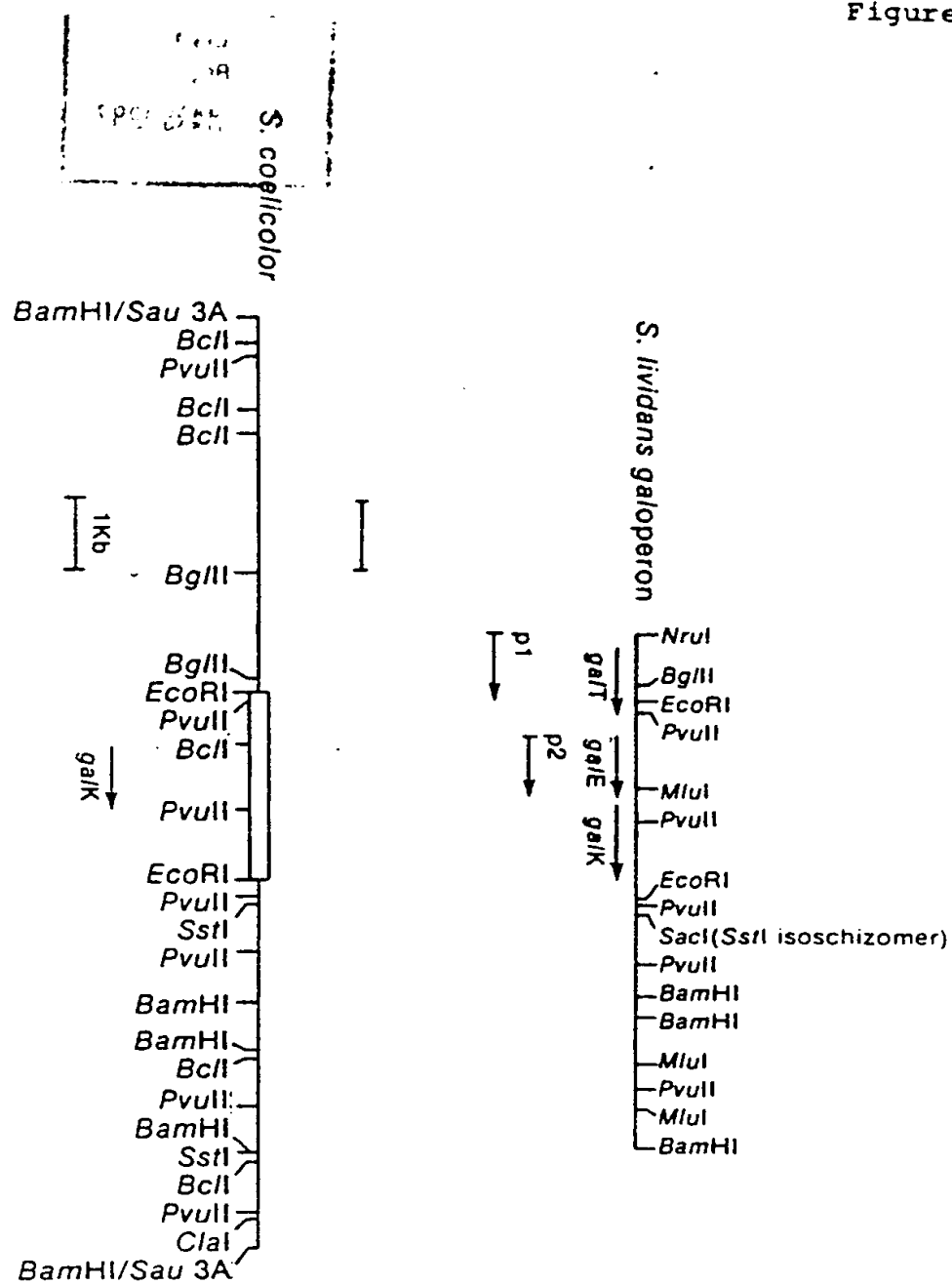


Figure 2

Figure 3







Europäisches Patentamt
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Office européen des brevets

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(54) **The gal operon of streptomyces.**

(57) A recombinant DNA molecule comprising the *Streptomyces gal* operon *galK* gene; *galE* gene; *galT* gene; P1 promoter; P2 promoter; P2 promoter expression unit; P1 promoter regulated region, or the entire *Streptomyces gal* operon is prepared.

EP 0 235 112 A3



European Patent
Office

EUROPEAN SEARCH REPORT

0235112

Application Number

EP 87 87 0026

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
D,A	GENE, vol. 40, no. 2/3, 1985, pages 191-201, Elsevier Science Publishers, Amsterdam, NL; M.E. BRAWNER et al.: "Characterization of Streptomyces promoter sequences using the Escherichia coli galactokinase gene" ---		C 12 N 15/00 C 12 N 1/20
D,A	NUCLEIC ACIDS RESEARCH, vol. 13, no. 6, 1985, pages 1841-1853, IRL Press Ltd, Oxford, GB; C. DEBOUCK et al.: "Structure of the galactokinase gene of Escherichia coli, the last (?) gene of the gal operon" ---		
A	EP-A-0 187 630 (SMITHKLINE BECKMAN CORP.) ---		
A	ABSTRACTS OF THE ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 84, no. 0, 1984, abstract no. H98; W. BURNETT et al.: "Transcriptional and translational regulatory elements in the streptomyces - lividans beta-gal operon" -----		
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			C 12 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 02-08-1988	Examiner PULAZZINI A.F.R.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

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